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Vol. V, Part IV August, 1935

THE INDIAN JOURNAL OF AGRICULTURAL SCIENCE

Issued under the authority
of
The Imperial Council of Agricultural Research

Published for
THE IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH

Annual subscription
Rs. 15 or 24s.
inclusive of Indian Postage

Price per part
Rs. 3 or 5s. 3d.
inclusive of Indian Postage

PUBLISHED BY MANAGER OF PUBLICATIONS, DELHI
PRINTED BY THE MANAGER, GOVERNMENT OF INDIA PRESS, NEW DELHI
1935

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ORIGINAL ARTICLES

HELMINTHOSPORIUM DISEASES OF BARLEY AND THEIR CONTROL

BY

ERRATA.

Page 480—

Heading of Table XIX, *for* the words "*percentage of death seedlings*" *read* *percentage death of seedlings*".

Page 485—

Contents, item VIII, *for* the word "PHYTOPLTHORA" *read* "PHYTOPHTHORA".

Page 530, fig. 1, *for* "10" at the left margin *read* "+10".

Page 543—

Last but one line, *delete* bracket before the word "*Sebastiania*".

(ii) Net blotch 452
(iii) Stripe disease 452
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I. Introduction

Three species of *Helminthosporium* are generally responsible for diseases of barley. In the seedling stage many plants succumb to the foot and root-rot diseases which these fungi cause and in the mature crop these fungi are responsible for blotches and spots on leaves, thus lowering photosynthetic activity of the plants by reducing the leaf area. In the barley growing tracts of Northern India, *H. sativum* P. K. & B., which causes a foot and root-rot disease in the seedling stage and leaf blotches later, is the more common pathogen. *H. teres* Sacc. has also been found to attack some types of barley. *H. gramineum* Rabh., which causes the severe stripe disease in barley in Europe and Northern America, is less common on the whole, though in the United Provinces of Agra and Oudh, it causes some damage.

H. sativum has, in recent years, been found in an epidemic form causing severe foot and root-rots at Pusa and also the blotch disease, leading to reduction in crop yields. The percentage of seedlings killed and the leaf area destroyed in mature plants have shown a good deal of variation in different types of Pusa barleys as well as in other Indian and foreign types grown at Pusa. The variation has not only been from type to type but also from season to season in the same type. The degree of attack has also varied from one locality to another, from plot to plot and sometimes also in different parts of the same plot. It has also been noted that some types of Pusa barleys are resistant to a certain extent, while others have shown high susceptibility, though none of them have been completely immune. The present investigations were undertaken in order to determine the varietal resistance of the Pusa types of barley to this species of *Helminthosporium* and the effect of certain fungicides in controlling the foot-rot and root-rot phases of the disease, and the data collected during the past four years are discussed in this paper.

II. Symptoms

These three *Helminthosporium* species cause certain definite and distinct symptoms in the barley plants.

(*i*) *Spot blotch*

H. sativum is the most destructive of the three species of *Helminthosporium* and it causes the foot and root-rot, the seedling blight, the leaf-spot, the head blight and the discoloration of seed (Plate XVIII). It stunts the growth of the barley plant, retards the development of roots and thus weakens its standing power. Even seed germination is lowered and finally causes appreciable loss in yield. The disease thus is manifested in two phases :—

(*a*) Primary infection due to the infection from the seed causing foot-rot and root-rot of seedling.

HELMINTHOSPORIUM SATIVUM

Six seedlings of barley showing various stages of 'foot-rot' and 'root-rot.' Leaves show spot formation (natural size).

(*b*) Secondary infection due to the conidia borne on the primary spots and causing fairly well defined, more or less longitudinal spot formation on all aerial parts on account of which it is called ' spot blotch '.

(*a*) *Primary infection* (*Foot-rot and root-rot*).—The first symptom of the disease in the field becomes apparent during the seedling stage of the barley plant. The seedlings show the appearance of blight very much like damping off. When severely attacked, the plants are killed at the ground level. A large number of diseased seedlings fail even to come out of the soil and the roots and the young shoots of such diseased seedlings show deep brownish discoloration, become brittle, and rot. The injury to the root is brought about by a rather limited number of local lesions which kill the root tips. When these lesions occur back from the tip they some times cut off portions of the root as well. Very often the emerging roots get infected and rot. In other cases the root system is fairly well developed and may be free from the disease or may be slightly attacked, but the stems show brownish discoloration at the foot and are soon destroyed. Sometimes the seedlings produce a few leaves but as the roots are attacked by the fungus the plants soon die. Severely infected seeds do not germinate but if they do germinate the seedlings generally do not emerge from the ground level, or sometimes only the tips of the leaves push out before the seedlings are killed. Infected plants are of course distinctly stunted. When such plants are up-rooted they show poor development of the root system, which has a brownish discoloration in parts due to the attack of the fungus. Very often the roots become so brittle and decayed that an attempt to pull them up causes them to break at the crown.

(*b*) *Secondary infection*.—The secondary phase of the disease is manifested by the appearance on the leaf blade of spots varying from 0·5 to 15 mm. in width and from 2 to 30 mm. in length. The blotches are oval or irregular, usually brown to dark brown and the colour fades at the margin of these blotches in the form of yellow zones which gradually merge into the green of the surrounding tissue. The coalescence of several spots results in the formation of big irregular spots. Often, as the fungus progresses into the leaf tissue, the spots become darker and finally reddish in colour, forming short, narrow, dark brown streaks. Blotches appear on the leaves of the seedlings as well as on those of the grown-up plants. The lower leaf blades are first affected and the blotches multiply until scores of them appear on one foliar organ and a considerable portion of the leaf tissue becomes involved. The entire blades are often affected to such an extent that they curl up and cause the death of the plant.

The fungus also forms spots in the stem especially at the nodes, attacks the inflorescence and may be found on glumes and not infrequently on the seed, sometimes causing the entire head or portion of it to be blighted. The diseased kernels usually are readily distinguished because of the dark brown discoloration at the germ end and such seeds when placed in a moist chamber give rise to a woolly mycelial growth within a day or two. Such severely affected seed occurs in years

when the disease is in an epidemic form and shows great reduction in the percentage of seed germination. In the case of very severe attacks the ovary is infected and under such conditions no seed is formed; whatever seed is set is badly shrunken and incapable of normal germination.

On the plots in which barley is grown year after year the disease is worst during the seedling stage and *H. sativum* has been isolated from such soil samples. This shows that the organism may live in the soil as well as in and upon infected barley grains. When the diseased kernels are planted, the fungus becomes active upon the germination of the seed, penetrates into the young root and leaves and causes reddish-brown to dark-brown spots.

(*ii*) *Net blotch*

The disease caused by *H. teres* is recognised by the appearance on the leaf blade of dark brown spots or streaks which at first are about 1 mm. in diameter but which, later, increase considerably in size in a longitudinal direction. The most distinctive feature of the species is the discoloured area involved in the net-blotch lesions. This characteristic "netting" of the discoloured area on leaves, sheaths, glumes, spikelets and grains distinguishes this blotch from the spot-blotch disease. Further, it does not cause splitting of the leaves which is so common in the stripe disease. The straw at harvest is dull brown and lacks strength. This disease does some damage to barley in India, and is restricted to only a few types and does not occur on local or on other exotic barley types grown at Pusa. The net-blotch disease, is common in the United Provinces and in Nepal but no report of any serious damage has hitherto been recorded.

This fungus is also known to cause foot-rot and root-rot in other countries but it has never been noticed at Pusa.

(*iii*) *Stripe disease*

The disease caused by *H. gramineum* is characterised by the occurrence of straw-coloured longitudinal streaks on the leaf blades, the typical stripe of the lesions. Another marked symptom of this disease is the failure of the ear to set seed normally. The disease first makes its appearance in the form of small yellowish spots on the leaves, later extending into long parallel streaks. The streaks become dark-brown or reddish brown and the whole leaf gradually dries up. The leaf blades are attacked as they develop, and sometimes the ear does not emerge from the sheath; if it does, it remains erect and stunted and the awns often become twisted and bent. The affected heads turn greyish-brown and the grains within are usually shrivelled. As the diseased portion of the blade within the yellowish green stripes dies, a splitting or shredding of the entire blade occurs. Dead leaves and plants soon crinkle and ultimately die. Fortunately, this serious disease of barley is not very common in India.

III. Host range and strains of *H. sativum*

H. sativum is not restricted to barley and is vigorously parasitic on several grasses, according to Stevens [1922] and Christensen [1926]. It has also been found to form spots on paddy and maize at Pusa and has been successfully inoculated in India on wheat, oats, sorghum, sugarcane, *Setaria italica and Panicum frumentaceum* by Mitra [1931, 1]. On all these hosts this organism is found to produce distinct spots on which fructifications are formed. The longevity of spores of *H. sativum* may extend to at least four years under Indian conditions. The organism can live as a facultative saprophyte on dead and dying plants. Its greatest luxuriance, however, is attained on the foliage of wheat and barley. There are several strains of this fungus but the most common are those on barley and wheat. The wheat strain is as vigorous as the barley one and both the strains are capable of infecting either of the two hosts.

Mitra [1931, 2] obtained several saltants of this fungus in culture media and some of these were later isolated from the host plants. This demonstrates that the saltants which arise in culture also occur under natural conditions. A study of the saltants of these two strains from wheat and barley has established the fact that the barley strain is capable of giving rise to saltants of the wheat strain type and *vice versa* [Mitra ; 1931, 2].

IV. Temperature relationship of *H. sativum*

Detailed studies in the morphological characters of the barley and wheat isolates of *H. sativum* have been recorded by the senior author [Mitra ; 1931, 2]. The rate of growth of the two strains at different temperatures has also been studied on different media and the general relation between temperature and the linear growth is shown in Figs. 1 to 4. Figs. 1 and 2 give the rate of spread of the barley strain on Brown's synthetic agar and Brown's synthetic starch agar over series of temperatures. Figs. 3 and 4 give a comparative picture of both barley and wheat strains on three media.

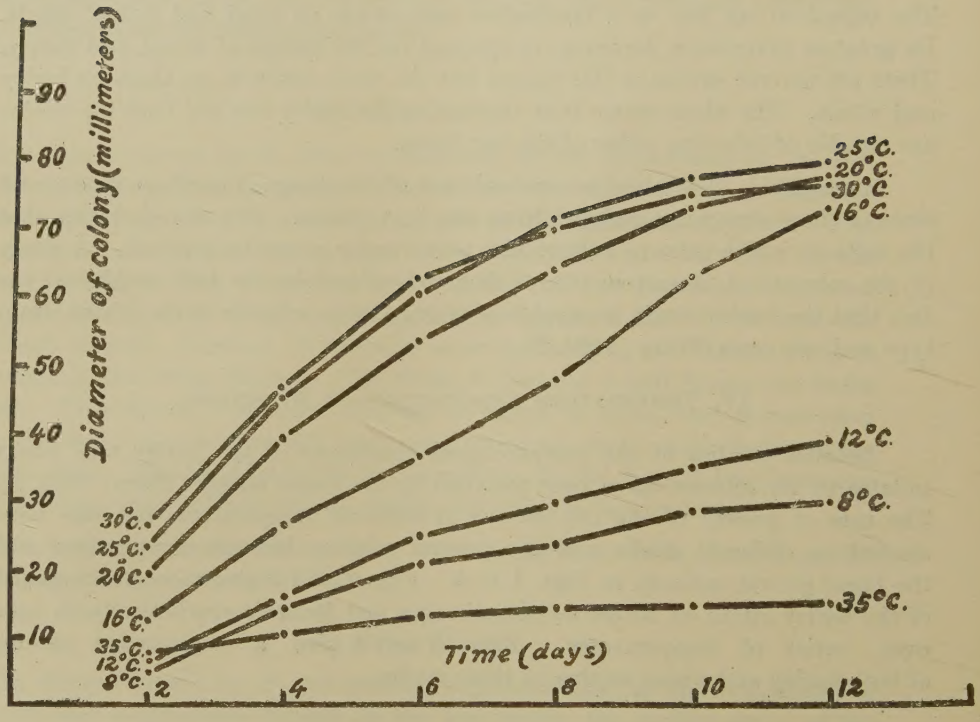

Fig. 1. Temperature relationship of *H. sativum* P. K. & B. (barley) on Brown's synthetic agar

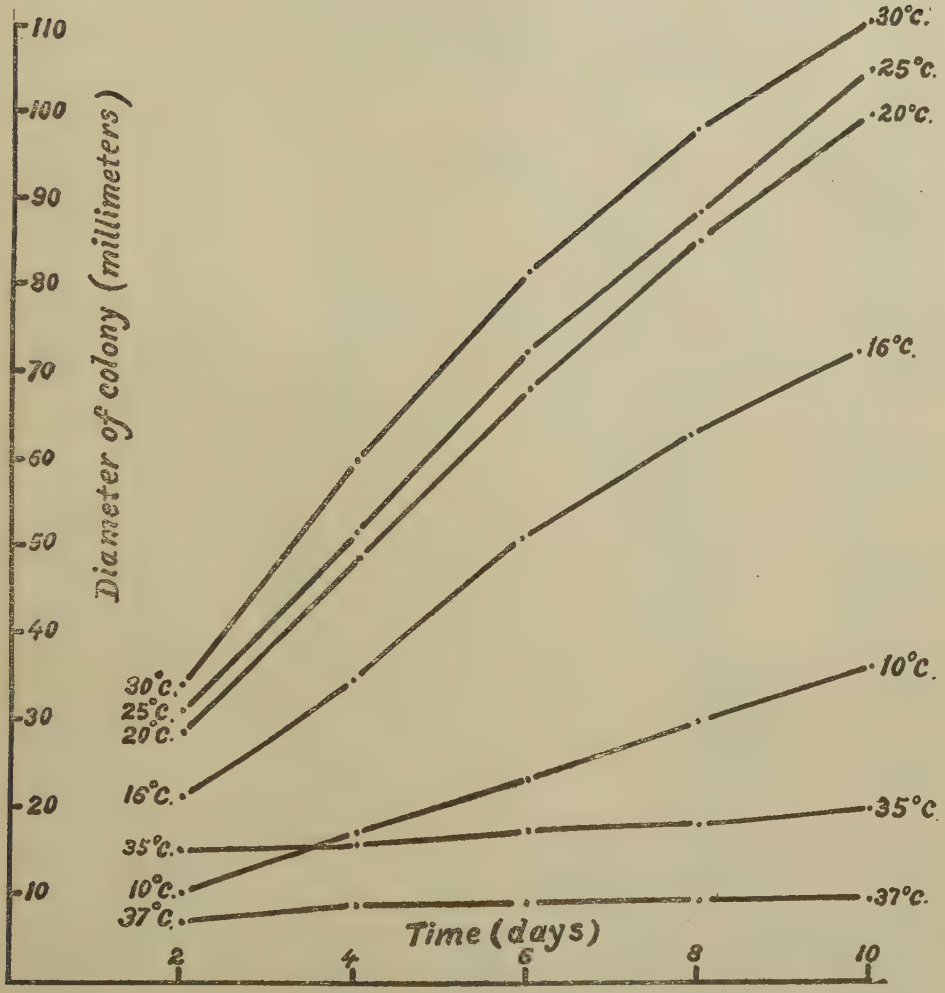

Fig. 2. Temperature relationship of *H. sativum* P. K. & B. (barley) on Brown's synthetic starch agar

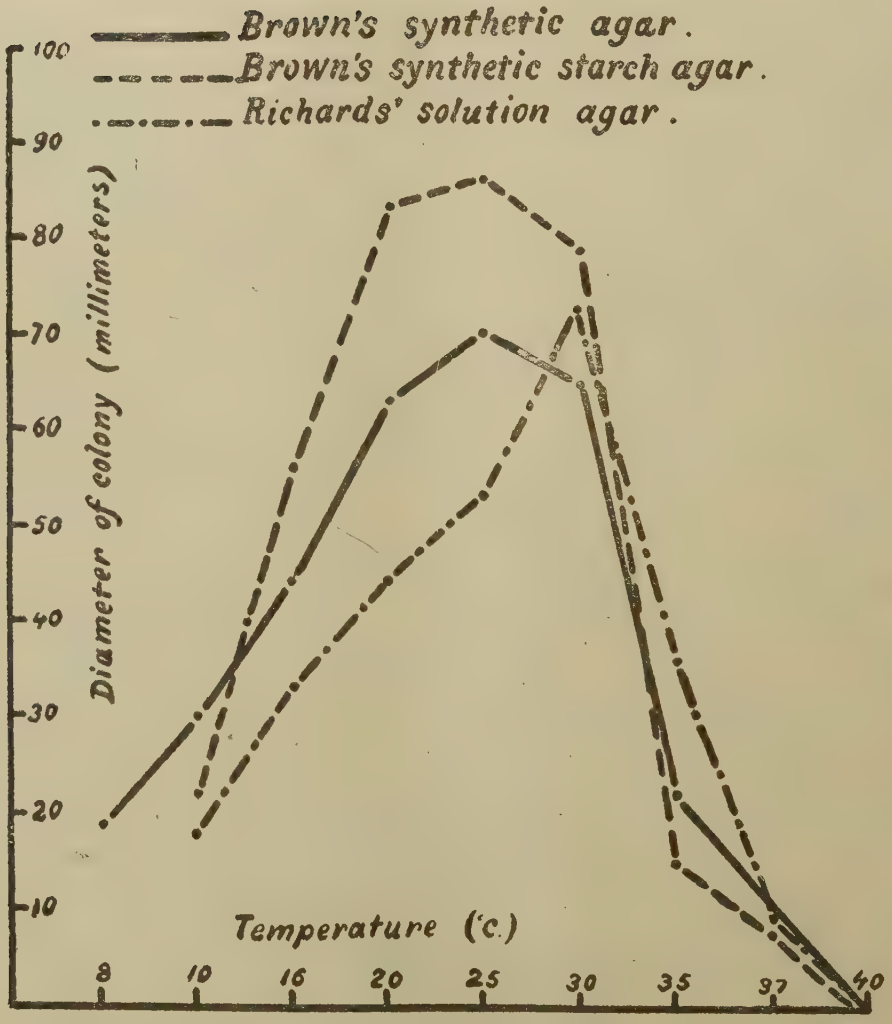

Fig. 3. Eight days' growth of *H. sativum* (wheat) on Richards' solution agar at various temperatures

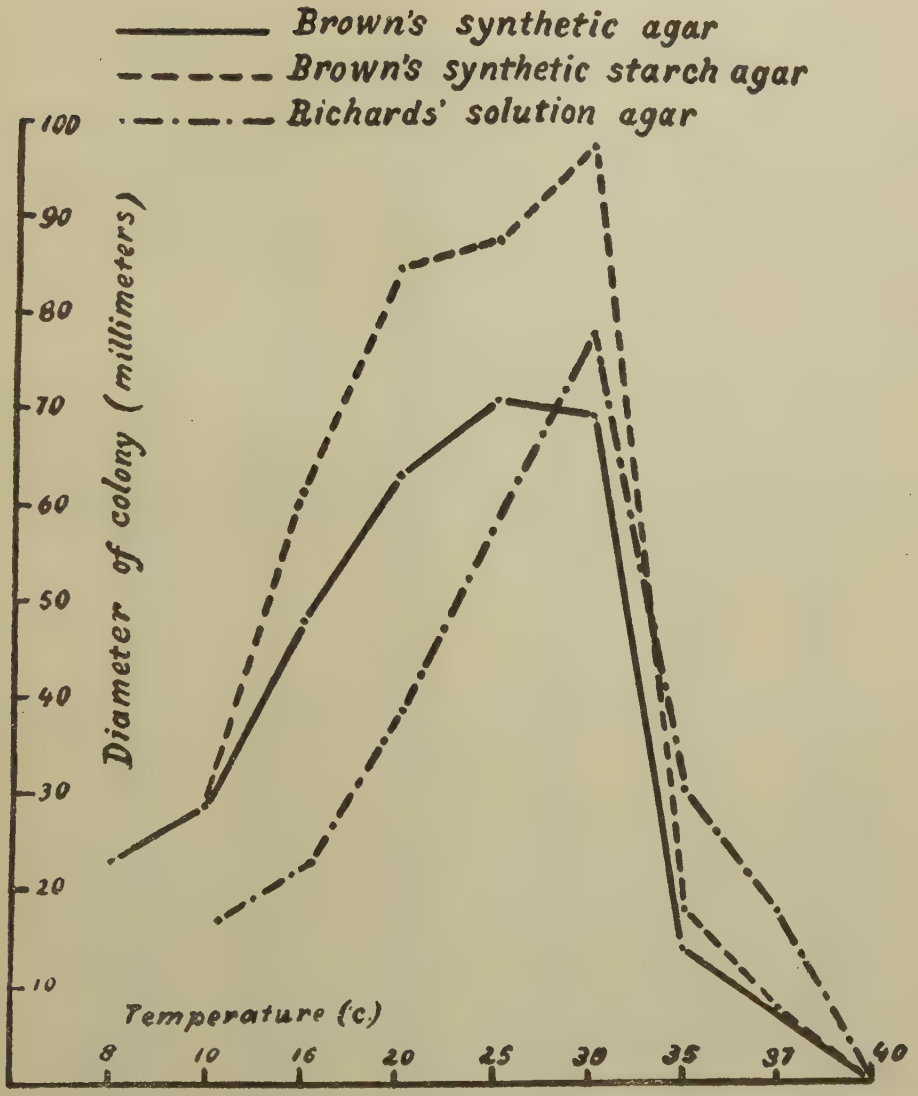

Fig. 4. Eight days' growth of *H. sativum* (barley) on Richards' solution agar at various temperatures

The optimum temperature for the two strains of *H. sativum* on different media is :—

TABLE I

Strain	Brown's agar	Brown's starch agar	Richards' agar
Wheat . . .	25°C.	25°C.	30°C.
Barley . . .	25°C.	25-30°C.	30°C.

From these figures we conclude that the optimum temperature for the growth of *H. sativum* lies between 25 and 30°C. Dosdall [1923] in her studies with the American strains of this fungus found the optimum between 24 and 28°C.

V. THE METHOD EMPLOYED IN DETERMINING THE PERCENTAGE OF LEAF AREA DESTROYED

In preliminary studies the amount of damage done to the leaf area was obtained by using comparative but arbitrary terms such as very slight, slight, fair and bad to represent the degrees of attack. These standards, however, are liable to alteration from season to season and from observer to observer. Personal errors thus crept in and a correct estimate could hardly be obtained. In order to overcome this difficulty a standard chart was prepared following Tehon [1927], in which leaf blades with 2, 5, 10, 15, 20, and 30 per cent area destroyed by the fungus, were diagrammatically represented. Such a chart is shown in Plate XIX.

The percentage of the leaf area destroyed in any type of barley was estimated by collecting the second and fourth leaves from a number of random plants in that type and then classifying the leaves according to the standard chart. A frequency distribution was thus obtained and the average value calculated as shown in Table II.

PLATE XIX.

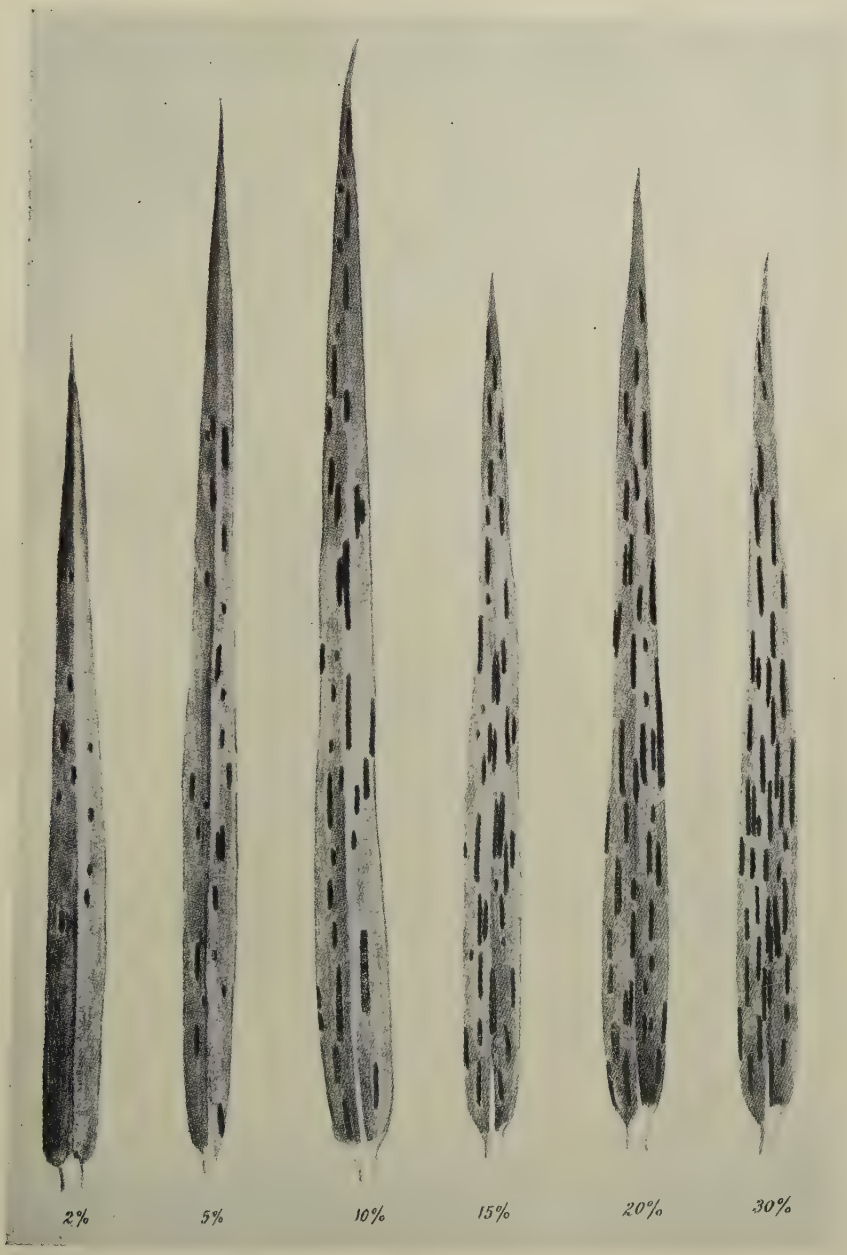

Chart for determining the percentage of leaf area destroyed.

TABLE II

Distribution of leaves of barley type 21 according to the percentage of leaf area destroyed

Seed treatment before sowing	Replication	Percentage of leaf area destroyed								
		0	2	5	10	15	20	30	Total no. of leaves	Average damage
Untreated—control	1	23	33	5	..	..	..	..	61	1.4
	2	38	38	3	..	..	..	..	79	1·1
	3	26	40	5	..	..	..	..	71	1·4
	4	20	35	7	3	..	..	..	65	2·0
	5	24	40	2	..	..	..	..	66	1·3
	6	30	39	6	1	..	..	..	76	1·5
	7	15	20	16	1	..	..	..	52	2·5
TOTAL .	..	176	245	44	5	..	..	..	470	Mean 1·6170 ±0·0952
Disinfected with uspulun (universal)	1	46	7	..	..	..	..	..	53	0·26
	2	103	..	..	..	..	..	..	103	0·00
	3	97	..	..	..	..	..	..	97	0·00
	4	76	..	..	..	..	..	..	76	0·00
	5	126	..	..	..	..	..	..	126	0·00
	6	107	2	..	..	..	..	..	109	0·03
	7	37	1	..	..	..	..	..	38	0·05
TOTAL .	..	592	10	..	..	..	..	..	602	Mean 0·0332 ±0·0040

Mean difference = 1.5838 ± 0·0953

The data presented as an illustrative example in the above table have reference to the method of treatment which is to be hereafter explained and have been cited here to show how average leaf areas destroyed are to be calculated.

The mean difference of 1·5838 ± 0·0953 per cent is statistically significant and denotes that a definite advantage has been gained in reducing the extent of the disease by disinfecting the seed with uspulun before sowing. This method of determining the leaf area destroyed by species of *Helminthosporium* affords a reliable estimate and is amenable to comparisons being made between different types in the same or in different seasons.

VI. Effect of environmental factors on disease development

Observations made during the last four years indicate that *H. sativum* is present every year in practically every field of barley at Pusa and that it is more destructive in some years than in others. Table III shows the percentage of leaf-area destroyed by *H. sativum* and *H. teres* in twenty-four types of barley [Bose, 1931] and the local country type during 1930-31, 1931-32, 1932-33 and 1933-34, and brings out the fact that there is a wide variation in the virulence of the disease on different types of barley from season to season. It will also be noticed that *H. sativum* attacks most of the Pusa barley types, whereas *H. teres* attacks only certain types which have been selected from samples originally collected from different parts of India [Bose, 1931]. The local type usually grown in the neighbourhood of Pusa is less susceptible to *H. teres*. In addition to the data presented in Table III the writers have observed the disease due to *H. sativum* in several areas in the neighbourhood of Pusa and have noted great variation in the incidence of the disease from year to year, from plot to plot and even in different parts of the same plot. This perhaps is due to differences in soil moisture, temperature, etc. Thus, it is apparent that certain ecological factors influence profoundly the severity of the disease. The fungus manifestly enters but remains quiescent in the host tissues when conditions are unfavourable preventing the pathogen from becoming destructive. Under favourable conditions, however, secondary infection may occur repeatedly till harvest.

Observations show that heavy dew and rain together with high temperature are conducive to foliage and spike infections. Infection can take place at any time of the season. The fungus has a wide range of temperature relationship and the spores are capable of germination even at 8°C. or at 33-35°C., the optimum temperature being between 25 and 30°C., that is, nearabout 28°C. As already shown by the senior author [Mitra ; 1931, 1] the higher temperatures are most favourable for the growth of the fungus, for spore germination and infection and generally for the development of the disease. He found that the amount of infection considerably increases as the temperature is raised from 20°C. to 30°C. The fungus can remain dormant in the host tissue for a long time under conditions unfavourable for its spread. At the time barley is sown at Pusa, the mean atmospheric and soil temperatures are nearer the optimal limits of the pathogen and hence considerable damage is caused during the seedling stage. The mean temperatures then fall and the fungus is inactive during the winter months, but with the approach of the spring at the time of heading out the atmospheric temperature again rises and the disease increases causing a good deal of secondary infection. The full effect of the destructive nature of the fungus becomes apparent when the crop is mature and is about to be harvested. Early varieties naturally escape the disease due to the comparatively lower temperatures prevailing at the time they mature,

It has been noticed that *H. gramineum* is either absent or very rare at Pusa and in its vicinity, and occurs only during certain years when a foreign type is grown at Pusa, *e.g.*, ' Cape barley' grown during 1921 in the Farm was very severel attacked by ' stripe disease ' and the whole crop had to be burnt. This rare occurrence of ' stripe disease ' is possibly due to the fact that temperatures at Pusa are not suitable for the growth of this organism. Johnson [1925] found that low soil temperature favours infection of barley by *H. gramineum* and that very little ' stripe disease ' occurs at temperatures higher than 20°C. Such low temperature does not occur at Pusa either at the time of sowing or harvesting which may account for the absence of the disease from Pusa.

VII. Epidemiology of the disease during 1930-34 on various types of barley

The percentage of leaf area rendered ineffective by *H. sativum* and *H. teres* on barley varieties grown at Pusa from 1930 to 1934 is shown in Table III. These percentages are based on an estimation from about 200 leaves in each type.

Table III

The percentage of leaf area rendered ineffective by Helminthosporium teres *and* H. sativum *on 24 types of barley during 1930-34*

Type	1930-31			1931-32			1932-33			1933-34		
	Attacked by		Combined infection	Attacked by		Combined infection	Attacked by		Combined infection	Attacked by		Combined infection
	H. teres	*H. sativum*		*H. teres*	*H. sativum*		*H. teres*	*H. sativum*		*H. teres*	*H. sativum*	
1	1.8	4.1	5.9	1.0	2.6	3.6	...	0.26	0.26	...	4.6	4.6
2	...	3.0	3.0	...	1.2	1.2	...	1.3	1.3	...	13.1	13.1
3	1·2	1.2	2.4	...	1.4	1.4	...	2.6	2.6	...	16.5	16.5
4	6·3	...	6.3	...	5.2	5.2	0.71	2.14	2.85	...	14.8	14.8
5	12·1	...	12.1	1.0	3.9	4.9	0.81	2.44	3.25	1.1	11.2	12.3
6	27·9	...	27.9	23.2	...	23.2	20.45	...	20.45	20.7	3.2	23.9
7	21·2	...	21.2	25.4	...	25.4	14.4	...	14.4	10.1	1.1	11.2
8	10·6	4.5	15.1	17.5	6.1	23.6	3.9	4.0	7.9	14.2	3.1	17.3
9	...	8.4	8.4	...	12.5	12.5	...	6.75	6.75	...	22.6	22.6
10	1.3	10.8	12.1	1.6	14.2	15.8	1.1	4.95	6.05	1.3	8.6	9.9
11	8.2	1.0	9.2	4.5	10.1	14.6	3.1	1.0	4.1	1.0	16.0	17.0
12	5.9	3.7	9.6	4.8	4.3	9.1	0.32	0.98	1.3	1.8	9.0	10.8
13	7.5	0.9	8.4	3.0	2.7	5.7	0.6	3.45	4.05	1.5	11.0	12.5
14	7.1	1.1	8.2	1.3	5.7	7.0	...	3.55	3.55	...	13.5	13.5
15	1.2	11.0	12.2	...	5.5	5.5	...	1.6	1.6	...	12.5	12.5
16	...	7.3	7.3	...	4.1	4.1	...	1.35	1.35	...	16.8	16.8
17	6.0	...	6.0	0.5	4.7	5.2	...	0.13	0.13	...	9.3	9.3
18	12.2	...	12.2	15.0	...	15.0	2.7	2.8	4.15	1.0	8.4	9.4
19	15.9	...	15.9	20.1	...	20.1	3.25	0.55	3.8	3.1	13.9	17.0
20	...	9.3	9.3	...	4.8	4.8	...	2.55	2.55	...	9.5	9.5
21	17.7	...	17.7	4.0	3.1	7.1	...	0.22	0.22	...	12.7	12.7
22	14.5	...	14.5	5.2	5.1	10.3	0.27	2.43	2.7	1.1	12.8	13.9
23	13.4	...	13.4	17.9	...	17.9	8.15	...	8.15	7.0	7.4	14.4
24	9.2	...	9.2	3.6	4.0	7.6	...	1.35	1.35	...	4.9	4.9
Country (local).	...	5.9	5.9	...	1.9	1.9	...	0.22	0.22	...	8.4	8.4

H. teres was not found in a virulent form up till 1930 but during that year it seems to have been introduced in the Botanical Section, Pusa, through some imported varieties, probably from England or from Nepal. Since then it has established itself and has proved very harmful to certain varieties as will be seen from the above table. During 1930-31 the attack of *H. teres* was bad on types 6, 7 and 21 and some others. Types 7, 12, 17 and 21 were also grown in another field but away from the source of infection, and there was no trace of *H. teres* in them. This fact supports the view that the organism has been introduced.

From Table III it will further be noticed that several types such as types 3, 4, 5, 11, 14, 17, 21 and 24 which got infected during 1930-31 with *H. teres* were either free from or were only lightly attacked by this species but the disease persisted in types 6, 7, 8 and a few others.

The data in Table III show that under the atmospheric conditions at Pusa none of the twenty-four types evolved by selection at this place are completely immune to the disease. The different types however show various degrees of resistance.

The total combined attack on various types varied from year to year. Table IV gives indications of the range of the percentage of leaf area destroyed by *H. teres* and *H. sativum* during the last four years.

TABLE IV

The range of percentage of leaf area destroyed by species of Helminthosporium *on 24 Pusa types of barley*

Organism	Percentage of leaf area destroyed			
	1930-31	1931-32	1932-33	1933-34
H. teres	0 to 27.9	0 to 25.4	0 to 20.45	0 to 20.7
H. sativum	0 to 11.0	0 to 14.2	0 to 6.75	1.1 to 22.6
Combined effect	2.4 to 27.9	1.2 to 25.4	0.13 to 20.45	4.6 to 23.9

From the above table it will be noticed that the intensity of the disease caused by *H. sativum* was less during 1932-33, but great in 1933-34 when it appeared in several varieties of barley and was responsible for the shrivelling and discoloration of the kernels. Spots extended even on to the awns, and in several cases the seed formed was totally destroyed.

The comparative virulence of *H. sativum* during the last four years on some of the barley types can be judged by an examination of figures for types 3, 4, 5, 9, 14, 16, 19 and 21 in Table III. It will be seen that the attack was most severe in the season 1933-34. The percentage of leaf area destroyed in these types varied in the above-mentioned types from 0 to 1·2 in 1930-31 to 11·2-22·6 in 1933-34,

VIII. Treatment of seed by fungicides to control foot-rot and root-rot and secondary infection

(*i*) *Experiments carried out during 1930-31*

With a view to study the effect of various treatments in checking the disease caused by *H. sativum* and testing the resisting power of certain types of Pusa barleys, four types which showed different degrees of resistance during the previous year were selected for the experiment in 1930-31. The attack of *H. sativum* on these four types during 1929-30 was the same as in Table V.

Table V

H. sativum—*Comparative infection of leaf area during 1929-30*

Date	3-12-29	22-12-29	6-1-30	21-1-30	5-2-30	20-2-30	5-3-30
Type							
7 . . .	S	S	S	F	F	F	B
12 . . .	S	S	S	F	F	F	F
17 . . .	S	S	S	S	S	S	S
21 . . .	VS	VS	VS	VS	S	S	S

Where VS = Leaf infection very slight.
S = Leaf infection slight.
F = Leaf infection fair.
B = Leaf infection bad.

These four types of barley were sown in 96 plots and each treatment for each variety had six replications. Each plot had sixty plants spaced one foot apart.

The following seed treatments were given before sowing :—

A—Control seed sown without any treatment.

B—Seed soaked in water for one hour before sowing.

C—Seed infected heavily with spores of *H. sativum* suspended in water.

D—Heavily infected seed disinfected with a 0·01 per cent solution of mercuric chloride for ten minutes.

Separate germination tests showed that the seed of all the four types under different treatments was almost cent per cent viable.

Death of seedlings due to H. sativum.—A careful weekly record was maintained of the number of seeds which germinated or which died of foot or root-rot caused by *H. sativum*. In some cases a number of seedlings were found to be damaged by white ants. There was no trace of *H. teres* in any of the types. In Table VI is shown the statistical analysis of the data, using Fisher's [1932] method.

TABLE VI

Analysis of variance of percentage death of seedlings due to H. sativum *and effect of seed treatment*

Due to	Degrees of freedom	Sum of squares	Mean squares	x—test	
				Observed value	Expected at $P=0.01$
Blocks	5	2482.0525	496.4105	13.9846	3.017
Varieties	3	10173.2477	3391.0826	95.5321	3.782
Treatments	3	1134.8895	378.2965	10.6572	3.782
Interaction					
Varieties × treatments	9	1222.5161	135.8351	3.8265	2.802
Residual error	75	2662.2592	35.4968	...	...
Total	95	17674.9650	186.0523	...	...

The differences of all possible mean values between the varieties and those between the different treatments are tabulated below. In all tables of mean differences used throughout the text the positive differences which are statistically significant at the one per cent level are given in thick type, whereas those that are significant at the five per cent level are printed in italics.

TABLE VII

Mean differences in the death of seedlings between varieties and between treatments

BETWEEN VARIETIES						BETWEEN TREATMENTS					
Varieties	T. 7	T. 12	T. 17	T. 21	Mean value	Treatment	A	B	C	D	Mean value
T. 7	...	—0·14	**24·06**	1·08	17·64	A	...	—0·29	2·74	—6·64	24·94
T. 12	0·14	...	**24·20**	1·22	17·50	B	0·29	...	3·03	—6·35	24·65
T. 17	—24·06	—24·20	...	—22·98	41·70	C	—2·74	—3·03	...	—9·38	27·68
T. 21	—1·08	—1·22	**22·98**	...	18·72	D	**6·64**	**6·35**	**9·38**	...	18·30

Critical difference at one per cent level—4·430.

It will be noted from the above table that during 1930-31 type 17 had about 41·70 per cent deaths in the seedling stage, whereas types 7, 12 and 21 had 17·64, 17·50 and 18·72 per cent respectively. Type 17 showed significantly higher percentage of deaths than all other types while the differences between the others were not significant.

Treatments A, B and C showed average death percentages of 24·94, 24·65 and 27·68 respectively which were not statistically different from each other but treatment D had only 18·30 per cent deaths which is significantly lower. This indicates that by disinfecting the seed before sowing, the destruction of the seedlings due to the disease was minimised. Thus type 17 was most susceptible to the attack of *H. sativum*, and treatment with mercuric chloride helped to reduce the disease during the seedling stage.

Destruction of leaf area by H. sativum.—When the crop was fully grown, the percentage of leaf area destroyed was estimated according to the method already mentioned. Table VIII gives the mean differences in the percentage of leaf area destroyed in the varieties and in the treatments.

TABLE VIII

Mean differences in the percentage of leaf area destroyed between varieties and between treatments

	BETWEEN VARIETIES					BETWEEN TREATMENTS					
Varieties	T. 7	T. 12	T. 17	T. 21	Mean value	Treatments	A	B	C	D	Mean value
T 7 .	...	2·44	—4·66	—4·54	6·03	A	...	—1·00	—0·19	—0·73	4·82
T. 12 .	—2·44	...	—7·10	—6·98	8·47	B	1·00	...	0·81	0·27	3·82
T. 17 .	4·66	7·10	...	0·12	1·37	C	0·19	—0·81	...	—0·54	4·63
T. 21 .	4·54	6·98	—0·12	...	1·49	D	0·73	—0·27	0·54	...	4·09

Critical difference is 0.4714 at the one per cent level and 0.3485 at the 5 per cent level.

It will be observed that types 7 and 12 were much more susceptible than types 17 and 21 which is in agreement with the results given in Table VI. Furthermore, type 12 is significantly more susceptible than type 7. The differences between types 21 and 17 on the other hand are not statistically significant.

Considering the percentage leaf destruction obtained in the four treatments, treatments A and C showed significantly higher damage than treatments B and D, but there was no statistical difference between treatments A (control) and C (infected). It is presumed that the incidence of secondary infection from other barleys, wheats or grasses had outweighed any differences due to initial infection.

It may be noticed that during 1930-31 the percentage destruction of leaf area in the four types under study has been inversely proportional to the deaths

in the seedling stage caused by foot or root-rot. This may be seen from the following figures taken from Tables VII and VIII :—

Type	Percentage destruction of leaf area	Percentage death of seedlings by foot-rot and root-rot
17	1·37	41·70
21	1·49	18·72
7	6·03	17·64
12	8·47	17·50

To sum up the results of 1930-31, the experiment indicates that, except in type 17, no increase in infection over the control is obtained by deliberate infection of the seed with spores prior to sowing. This is presumably due to either the soil or the seed already being infected heavily. Infection followed by disinfection reduces the percentage of death from foot-rot and root-rot to well below those of the control in the case of type 17 and to a lesser extent in type 21, while type 12 and type 7 are unaffected. Further, it appears that the more susceptible the variety to foot-rot and root-rot, the smaller is the percentage of destruction of leaf area in the surviving plants.

(ii) Experiments carried out during 1931-32

During 1931-32 four more types were added to the experiment made in the previous year for the determination of their susceptibility to *H. sativum*. In treatment D of this experiment formalin was used for disinfection instead of mercuric chloride. Infected seeds as in treatment C were disinfected with formalin, 1 : 320, or a solution made by adding one pint of formalin (guaranteed 40 per cent solution of formaldelyde) to 40 gallons of water. The seeds were soaked for twenty minutes in this solution and then were covered up for two hours for the formalin vapour to act. An additional treatment E was introduced in which untreated seed as in treatment A was disinfected with formalin in the manner explained above.

The eight types of barley were sown in 200 plots, each plot having 160 seeds. Each treatment was given five replications. Seedlings which died were removed and examined at regular intervals and recorded.

Death of seedlings.—The percentage of death in seedlings due to foot-rot or root-rot was uniformly high during this year and the results of this experiment are presented in Table IX.

Table IX

Mean differences in the death of seedlings between varieties and between treatments

Varieties	Between varieties								
	T. 1	T. 2	T. 5	T. 7	T. 12	T. 17	T. 20	T. 21	Mean value
T. 1	...	—0·42	2·99	2·84	—3·17	1·02	***4·91***	—4·57	46·66
T. 2 .	0·42	...	3·41	3·26	—2·75	1·44	***5·33***	—4·15	46·24
T. 5 .	—2·99	—3·41	...	—0·15	—6·16	—1·97	1·92	—7·56	49·65
T. 7 .	—2·84	—3·26	0·15	...	—6·01	—1·82	1·93	—7·41	49·50
T. 12 .	3·17	2·75	**6·16**	**6·01**	...	4·19	**8·08**	—1·40	43·49
T. 17 .	—1·02	—1·44	1·97	1·82	—4·19	...	3·89	—4·59	47·68
T. 20 .	—4·91	—5·33	—1·92	—1·93	—8·08	—3·89	...	—9·48	51·57
T. 21 .	***4·57***	***4·15***	7·56	7·41	1·40	***4·59***	9·48	...	42.09

Critical difference at one per cent level = 5·7132
Critical difference at 5 per cent level = 4·3472

Treatments	Between treatments					
	A	B	C	D	E	Mean value
A	...	—2·88	***3·50***	—8·54	—12·43	51·13
B	2·88	...	**6·38**	—5·66	—9·55	48·25
C	—3·50	—6·38	...	—12·04	—15·93	54·63
D	**8·54**	**5·66**	**12·04**	...	—3·89	42·59
E	**12·43**	9·55	**15·93**	***3·89***	...	38·70

Critical difference at one per cent level = 4·518
Critical difference at 5 per cent level = 3·437

The percentage of deaths in the seedling stage due to this disease was invariably very high this year in all the eight types of barley under investigation and ranged from 42·09 per cent in type 21 to 51·57 per cent in type 20. Statistical significance between any two mean values was, therefore, observed only in a few cases and any difference which was higher than the critical difference of 4·3472 per cent was taken as significant.

Although treatments A and B were not significantly different from each other in effect, they showed a higher percentage of deaths in their seedling stages than the disinfected series in treatments D and E. Treatment C had statistically a higher death rate than all other treatments, whereas treatments D and E were the least affected.

Destruction of leaf area.—The average percentage difference of leaf area rendered ineffective by the disease between the different types and the various treatments is shown in Table X.

TABLE X

Mean difference in the percentage of leaf area destroyed between varieties and treatments

Varieties	BETWEEN VARIETIES								
	T. 1	T. 2	T. 5	T. 7	T. 12	T. 17	T. 20	T. 21	Mean value
T. 1	...	1·76	5·00	14·22	6·74	4·66	6·34	4·61	0·14
T. 2	—1·76	...	3·24	12·46	4·98	2·90	4·58	2·85	1·90
T. 5	—5·00	—3·24	...	9·22	1·74	—0·34	1·34	—0·39	5·14
T. 7	—14·22	—12·46	—9·22	...	—7·48	—9·56	—7·88	—9·61	14·36
T. 12	—6·74	—4·98	—1·74	7·48	...	2·08	0·40	—2·13	6·88
T. 17	—4·66	—2·90	0·34	9·56	—2·08	..	1·68	—0·05	4·80
T. 20	—6·34	—4·58	—1·34	7·88	—0·40	—1·68	...	—1·73	6·48
T. 21	—4·61	—2·85	0·39	9·61	—2·13	0·05	1·73	...	4·75

Critical difference at one per cent level = 1·0896.
Critical difference at 5 per cent level = 0·8291.

Treatments	BETWEEN TREATMENTS					
	A	B	C	D	E	Mean value
A	...	0·02	0·60	*1·07*	0·74	5·07
B	—0 02	...	0·58	*1·05*	0·72	5·09
C	—0·60	—0·58	...	0·47	0·14	5·67
D	—1·07	—1·05	—0·47	...	—0·33	6·14
E	—0·74	—0·72	—0·14	0·33	...	5·18

Critical difference at one per cent level = 1·3242.
Critical difference at 5 per cent level = 1·0058.

It will be evident that barley type 7 suffered the most from secondary infection and showed an average destruction of 14·36 per cent of its leaf area. This value was statistically higher than that shown by any other type. The other six-rowed barleys, *viz.*, types 12, 20, 17 and 21, respectively, had 6·88, 6·48, 4·80 and 4·75 per cent of their leaf areas damaged by the fungus. Of the two-rowed barleys, type 5 was the worst affected having 5·14 per cent destruction, whereas types 2 and 1 had losses of only 1·90 and 0·14 per cent respectively. It may be added that type 1 is the earliest maturing Pusa barley and as such escapes secondary infection to a very great extent. These results again prove that the incidence of secondary infection, causing leaf destruction by *H. sativum*, brings about inappreciable differences between the control, the infected and the disinfected series. In other words, disinfecting the seed does not help in checking secondary infection in mature plants to any appreciable extent, though it helps in reducing the disease in the primary phase.

In the four types of barley that were studied in the previous year it was found that the percentage destruction of leaf was inversely proportional to the deaths caused by *H. sativum* in the seedling stage. This year, however, this fact was not corroborated as will be apparent from the following figures selected from Tables IX and X.

Type	Percentage destruction of leaf area	Percentage deaths of seedlings by foot-rot and root-rot
1	0.14	46.66
2	1.90	46.24
21	4.75	42.09
17	4.80	47.68
5	5.14	49.65
20	6·48	51.57
12	6·88	43.49
7	14·36	49.65

Effect of different treatments on the incidence of H. sativum *on barley types 7 and 21 in 1931-32.*—In addition to treatments A to E the results of which have already been enumerated above, four other disinfectants were tried this year in another set of experiments with types 7 and 21 only. The eight treatments used were :—

Northern portion of the field—

A. Control—seed sown dry in the usual way.

B. Seed soaked in water for one hour before sowing.

C. Seed infected heavily with spore suspension in water and sown.

D. Infected seed as in C disinfected with formalin (Formalin 1 : 320, or a solution made by adding a pint of formalin to 40 gallons of water. Seeds kept soaked for 20 minutes and then under formalin vapour for two hours).

E. Seed disinfected with formalin as above.

Southern portion of the field—

F. Control—seed sown dry in the usual way as in A.

G. Seed as in A disinfected with formalin as in D.

H. Seed as in A disinfected with uspulun (0·25 per cent solution for one hour).

I. Seed as in A disinfected with mercuric chloride (1 : 1000) for ten minutes and then washed in running water before sowing.

J. Seed as in A disinfected by dusting with sulphur at the rate of four ounces of fine sulphur (No. 120) per 60 lb. of seed.

Difference between the two types of barley

Type	Average percentage of leaf area destroyed	Average percentage of death of seedlings
7	13·24	41·96
21	4·78	36·16
Critical difference at one per cent level	0·87	2·42

Type 7 in both cases shows statistically higher casualties.

Difference between the various treatments

Treatment	Average percentage of leaf destroyed	Average percentage of death of seedlings
A	9·04	49·68
B	8·80	47·44
C	10·17	55·08
D	10·52	42·59
E	9·25	34·21
F	7·77	41·76
G	7·02	36·06
H	8·37	24·19
I	9·17	27·26
J	9·99	31·31
Critical difference at 5 per cent level	1·48	9·21
Critical difference at one per cent level	1·95	12·12

It will be apparent from the above table that as far as the destruction of leaf area is concerned the infected series C shows statistically higher destruction than F, G and H only, and that it is not significantly higher than the other treatments.

As regards the average percentage of seedling deaths, C (infected) is certainly the worst and treatments H, I, J the best. There is no statistical difference, however, between treatment C and either A or B. It may be concluded that the different disinfection treatments used in this experiment minimise the effect of the disease in barley.

In another experiment during 1931-32 seeds of 24 types of Pusa barleys were disinfected with formalin and uspulun to control the disease. The treatment was conducted as follows : (*a*) Control, no treatment ; (*b*) seeds disinfected with formalin as described before ; (*c*) seeds disinfected with uspulun (universal), 0·25 per cent solution for one hour. The extent of damage to the leaves was as shown below :—

TABLE XI

The extent of damage to leaf area in different types of barley in 1931-32

Damage	Control (No treatment)	Formalin treatment	Uspulun treatment
Very slight . . .	*Nil*	Two types—2, 3 . .	Six types—1, 2, 3, 4, 5, 17
Slight	Two types—2, 3 . .	Five types—1, 4, 5, 17, 21	Twelve types—11, 12, 13, 15, 16, 18, 19, 20, 21, 22, 23, 24.
Fair	Thirteen types—1, 4, 5, 12, 13, 14, 15, 16, 17, 20, 21, 22, 24.	Fifteen types—8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 22, 23, 24.	Five types—7, 8, 9, 10, 14
Bad	Nine types—6, 7, 8, 9, 10, 11, 18, 19, 23.	Two types—6, 7 . .	One type—6.

The above table confirms the previous results that uspulun treatment controls the disease to a certain extent and is more effective than formalin treatment.

Effect of various treatments on the yield of barley types

In 1931-32 the final yields of all the plots under the eight types of barley were taken and statistically analysed in order to see whether artificial infection reduced the yield and also whether disinfection with formalin increased it significantly. Table XII presents the yields in decagrammes obtained per plot.

TABLE XII

Yields in decagrammes of eight types of barley under five different seed treatments

Treatment	Replication	Varieties								Treatment totals
		T. 1	T. 2	T. 5	T. 7	T. 12	T. 17	T. 20	T. 21	
A . .	1	9	4	4	27	31	13	31	58	177
	2	45	4	4	94	103	45	99	145	539
	3	45	4	4	14	126	49	72	144	458
	4	63	18	13	76	117	81	112	148	628
	5	31	13	22	108	139	90	44	184	631
										2,433
B . .	1	31	13	4	58	81	9	72	67	335
	2	18	4	4	54	81	36	81	85	363
	3	45	9	13	112	139	58	108	166	650
	4	54	9	4	117	130	58	121	166	659
	5	58	4	22	54	72	22	63	144	439
										2,446
C . .	1	18	9	4	58	90	22	72	90	363
	2	58	9	9	67	144	36	45	63	431
	3	63	9	9	121	139	45	117	126	629
	4	67	13	9	94	108	67	67	144	569
	5	22	9	18	49	81	45	117	94	435
										2,427
D . .	1	27	13	4	58	81	54	76	103	416
	2	72	9	9	112	117	36	67	162	584
	3	90	27	4	121	157	81	112	157	749
	4	54	9	9	108	135	81	67	139	602
	5	31	9	4	72	117	45	117	81	476
										2,827
E . .	1	36	9	4	108	112	45	81	126	521
	2	45	13	9	117	135	58	108	193	678
	3	9	9	22	144	148	72	112	121	637
	4	31	22	13	108	189	90	108	139	700
	5	36	22	22	108	135	76	121	166	686
										3,222
Varietal totals .	..	1,058	273	243	2,159	2,907	1,314	2,190	3,211	13,355

The analysis of variance for these data is shown in the following table.

TABLE XIII

Analysis of variance

Due to	Degrees of freedom	Sum of squares	Mean square	Value of $\frac{\text{variance 1}}{\text{variance 2}}$	
				Observed	Expected at $P = \cdot 01$
Varieties	7	356143·435	50877·6335	116·394	2·802
Treatments	4	12368·550	3092·1375	7·074	3·320
Replications	4	29628·650	7407·1625	16·956	3·320
Interactions.					
Varieties × treatments	28	15610·690	557·5246	1·275	1·791
Treatments × replications	28	16897·050	603·4661	1·385	1·791
Residual error	128	55950·500	437·1132	...	...
Total	199	486598·875	2441·1953	...	...

It will be noticed that the mean square contributed by the varieties is the greatest and is followed by that for replications and treatments.

The differences in mean yields between the five treatments studied are shown below :—

TABLE XIV

Mean differences between treatments

Treatments	A	B	C	D	E	Mean yield
A	..	0·325	—0·150	*9·850*	**19·725**	60·825
B	—0·325	..	—0·475	*9·525*	**19·400**	61·150
C	0·150	0·475	.....	*10·000*	**19·875**	60·675
D	—9·850	—9·525	—10·000	..	*9·875*	70·675
E	—19·725	—19·400	—19·875	—9·875	..	80·550

Critical difference at one per cent level is 12·042 and at 5 per cent level is 9·163.

It is seen that the combined mean yields of all the eight types of barley under treatments A, B and C are 60·825, 61·150 and 60·675 decagrammes respectively and the difference between any two of these values is not significant. The mean yields of the disinfected series D and E are 70·675 and 80·550 decagrammes

respectively. These average values are statistically greater than those in the other treatments. Treatment E especially yielded the highest. Disinfection with formalin therefore has produced significantly higher yields in barley because of the fact that it controls the disease to some extent. This will be evident also from a comparison of the yields of each type under the different treatments.

TABLE XV

Mean yields in decagrammes of different types of barley under various seed treatments

Treatment	Barley types							
	1	2	5	7	12	17	20	21
A	193	43	47	319	516	278	358	679
B	206	39	47	395	503	183	445	628
C	228	49	49	389	562	215	418	517
D	274	67	30	417	607	297	439	642
E	157	75	70	585	719	341	530	745

Treatments D and E (disinfected series) show higher yields than the other treatments in most of the barley types under investigation.

(*iii*) *Experiments carried out during 1932-33 and 1933-34*

In these two years all the twenty-four types of barley evolved at Pusa were tried. During 1932-33 the seed was treated before sowing as follows :—

A. Control—no treatment.

B. Seed soaked in water for one hour.

C. Seed infected heavily with spore suspension in water.

D. Infected seed as in treatment C disinfected with uspulun (universal) for one hour.

E. Control seed as in treatment A disinfected with uspulun (universal).

During 1933-34 an additional sixth treatment, F, was given in which infected seed as in C was disinfected with ceresan at the rate of two ounces per bushel of seed. Each of the twenty-four types had six replications for each of the treatments during 1932-33 and seven during 1933-34.

The virulence of the disease as expressed both by deaths in the seedling stage as well as by the destruction of leaf area was definitely greater in 1933-34 than in 1932-33.

The average percentage loss due to seedling mortality and to destruction in leaf area in the twenty-four types of Pusa barley, irrespective of seed treatments, is shown in Table XVI. If the differences between the average values of any two types in this table are greater than the expected critical difference shown at the bottom of each column, the differences may be gauged to be statistically significant.

TABLE XVI

Average percentage loss in different types of Pusa barleys due to the attack of H. sativum

Type	Average percentage of seedling mortality		Average percentage of leaf area destroyed	
	1932-33	1933-34	1932-33	1933-34
1	17·64	12·46	0·01	3·32
2	17·90	14·86	0·46	8·00
3	21·93	14·60	1·59	9·78
4	23·59	16·87	2·27	7·14
5	28·39	21·06	2·74	8·50
6	12·61	13·81	13·37	14·22
7	11·10	11·45	8·26	17·46
8	12·15	11·92	5·81	15·37
9	10·47	11·47	1·31	18·36
10	11·81	11·96	1·00	14·47
11	14·05	12·70	3·39	11·33
12	8·62	14·90	0·46	11·14
13	17·86	20·23	0·63	17·41
14	22·82	27·24	0·71	13·68
15	20·67	17·40	1·92	18·29
16	8·62	18·47	0·35	10·99
17	9·49	15·05	0·01	7·64
18	13·39	20·95	0·75	5·23
19	10·93	10·90	0·80	12·11
20	13·40	11·33	0·37	10·93
21	16·44	10·12	0·22	8·45
22	10·75	16·38	0·39	11·38
23	15·45	14·44	0·89	14·45
24	19·78	22·05	0·23	4·18
General mean	15·386	15·526	1·998	11·823
Standard error of difference	1·908	1·380	0·224	0·306
Critical difference at one per cent level	3·740	3·555	0·576	0·775

The results are obvious and hence tables of differences are hardly necessary. It may be concluded that the various types of Pusa barley respond differentially to this disease and that the seasonal conditions play an important role in the incidence of the disease on the different varieties tried. Types 1 to 5 belong to the two-rowed species of barley and tend to show less destruction of leaf area than the other types which are six-rowed barleys.

Tables XVII and XVIII show the mean differences observed under various treatments in the two seasons, 1932-33 and 1933-34 in respect of percentage of seedlings which died and for the destruction of leaf area.

TABLE XVII

Average differences in percentage of loss in different seed treatments due to the attack of H. sativum*—1932-33*

Death in seedling stage							Destruction of leaf area						
Treatments	A	B	C	D	E	Mean value	Treatments	A	B	C	D	E	Mean value
A . . .	...	0·78	—0·14	—6·34	—5·07	17·54	A . .	...	—0·471	**0·771**	—0·013	—0·913	1·935
B . . .	—0·78	...	—0·92	—7·12	—5·85	18·32	B . .	—0·471	...	**0·300**	—0·484	—1·384	2·406
C . . .	0·14	0·92	...	—6·20	—4·93	17·40	C . .	—0·771	—0·300	...	—0·784	—1·684	2·706
D . . .	**6·34**	**7·12**	**6·20**	...	1·27	11·20	D .	0·013	**0·484**	**0·784**	...	—0·900	1·922
E . . .	**5·07**	**5·85**	**4·93**	—1·27	...	12·47	E . .	**0·913**	**1·384**	**1·684**	**0·900**	...	1·022

Critical difference at the one per cent level — 1·944

Critical difference at the one per cent level — 0·263

TABLE XVIII

Average differences in percentage of loss in different seed treatments due to the attack of H. sativum*—1933-34*

Death in seedling stage								Destruction of leaf area							
Treatments	A	B	C	D	E	F	Mean value	Treatments	A	B	C	D	E	F	Mean value
A . .	...	—0·31	—3·36	—10·19	—11·93	—15·51	22·41	A . .	..	**0·988**	—1·423	—0·709	—0·515	—4·001	12·767
B . .	0·31	...	—3·05	—9·88	—11·62	—15·20	22·10	B . .	—0·988	...	—2·411	—1·697	—1·503	—4·989	13·755
C . .	**3·36**	**3·05**	...	—6·83	—8·57	—12·15	19·05	C . .	**1·423**	**2·411**	...	**0·714**	**0·908**	—2·578	11·344
D . .	**10·19**	**9·88**	**6·83**	...	—1·74	—5·32	12·22	D . .	**0·709**	**1·697**	—0·714	...	0·194	—3·292	12·058
E . .	**11·93**	**11·62**	**8·57**	**1·74**	...	—3·58	10·48	E . .	**0·515**	**1·503**	—0·908	—0·194	...	—3·486	12·252
F . .	**15·51**	**15·20**	**12·15**	**5·32**	**3·58**	...	6·90	F . .	**4·001**	**4·989**	**2·578**	**3·292**	**3·486**	...	1·066

Critical difference at the one per cent level—1·771

Critical difference at the one per cent level—0·387

It will be noted from the two tables given above that artificial infection (treatment C) has hardly had any appreciable difference on the death of seedlings caused by this fungus, and as a matter of fact the percentage of deaths appeared to be somewhat lower than in the treatments A and B which were taken as controls. This reduction may possibly be due to the fact that at the time of artificial infection, which was done in a suspension of spores in water, the lighter and weak seeds which usually float on the surface of the water and which perhaps were more susceptible to the disease were inadvertently thrown away, and hence there was an elimination of some susceptible material and only healthy and plump seeds remained to be sown under treatment C.

The disinfected series D, E and F have definitely shown less damage both in the seedling stage as well as on the leaf area. Ceresan (treatment F) especially appears to have controlled the disease better than uspulun (treatments D and E). In 1932-33 the highest damage due to secondary infection was shown by treatment C (artificially infected) where the mean value worked out to be 2·706 per cent. The second and third places were taken by treatments B (seed soaked in water before sowing) and A (seed sown dry), respectively, with mean values of 2·406 per cent and 1·935 per cent respectively. In treatment D (artificially infected and then disinfected with uspulun) the mean leaf area infected by *H. sativum* was 1·922 per cent, a value which was not statistically different from the values obtained for treatments A, B and C. Treatment E, however, in which ordinary seed similar to that in treatment A was disinfected with the same strength of uspulun, showed only 1·022 per cent damage and this value was statistically less than that shown by the other treatments. This indicates that uspulun was effective in controlling the disease to an appreciable extent in treatment E but not so in treatment D because of the additional artificial infection given immediately before disinfection. Secondary infection appears to have reduced the variability due to the different treatments except F in 1933-34.

Considering the average percentage loss in treatment A very low correlations were found to exist between :—

1. Seedling deaths in 1932-33 and those in 1933-34 ($r = 0{\cdot}0980 \pm 0{\cdot}1364$),
2. Leaf area destruction in 1932-33 and that in 1933-34 ($r = 0{\cdot}0944 \pm 0{\cdot}1365$),
3. Seedling mortality and leaf area destruction in 1932-33 ($r = -0{\cdot}1018 \pm 0{\cdot}1362$),
4. Seedling mortality and leaf area destruction in 1933-34 ($r = -0{\cdot}0374 \pm 0{\cdot}1375$).

IX. Seasonal effect

That season exerts a great deal of influence on the incidence of *H. sativum* and consequently on the damage caused by this fungus to the barley plant is a fact which cannot be contested and has already been demonstrated by the results

enumerated in the preceding pages. A statistical proof of this can be obtained by analysing in detail the behaviour of types 7, 12, 17 and 21 during the four years 1930-31 to 1933-34.

In Table XIX is presented the complete analysis of variance of the percentage of deaths in the seedling stage and of the percentage leaf area destroyed by *H. sativum* respectively. It will be noted that for both these characters the largest figure for variance or mean square has been contributed by the variance due to seasons, and specially in the case of seedling mortality this figure is very pronounced and very much higher than that contributed by any other variables proving thereby that season exerts a great deal of influence in the variability of the disease affecting different types of barley.

TABLE XIX

Analysis of variance of percentage of death seedlings and leaf area destruction respectively due to Helminthosporium *from 1930-31 to 1933-34*

Due to	Degrees of freedom	Death of seedlings			Leaf area destruction			Expected ratio of $(S_1)^2/(S_2)^2$ at one per cent level
		Sum of squares	Mean squares $(S_1)^2$	Observed ratio— $(S_1)^2/(S_2)^2$	Sum of squares	Mean squares $(S_1)^2$	Observed ratio— $(S_1)^2/(S_2)^2$	
Blocks	4	496·8615	124·2154	7·142	27·60	6·90	11·696	3·320
Varieties	3	3029·3550	1009·7850	58·063	3494·22	1164·74	1974.234	3·782
Seasons	3	63463·9420	21154·6473	1216·392	4261·82	1420·61	2407·934	3·782
Treatments	3	3949·6472	1316·5491	75·702	31·36	10·45	17·713	3·782
Interaction :—								
Varieties × blocks	12	474·6170	39·5514	2·274	77·20	6·433	10·904	2·182
Treatments × seasons	9	3033·5833	337·0648	19·381	108·22	12·024	20·381	2·511
Seasons × blocks	12	2783·0570	231·9214	13·335	137·07	11·423	19·362	2·182
Treatments × blocks	12	905·6262	75·4688	4·339	38·44	3·203	5·429	2·182
Treatments × varieties	9	1037·8183	115·3131	6·631	152·43	16·951	28·708	2·511
Varieties × seasons	9	8648·7195	960·9688	55·256	886·81	98·534	167·015	2·511
Treatments × seasons × blocks	36	2439·5783	67·7661	3·897	182·90	5·085	8·619	1·000
Treatments × seasons × varieties	27	5025·1702	186·1174	10·702	438·52	16·241	27·528	1·791
Seasons × varieties × blocks	36	1331·5993	36·9887	2·127	427·16	11·866	20·113	1·000
Treatments × varieties × bolcks	36	2494·1535	69·2820	3·984	88·76	2·466	4·180	1·000
Residual error	108	1877·6672	$17.3858(S_2)^2$	..	63·37	$.59(S_2)^2$	..	..
Total	319	100991.3955	316.5874		10415.88	32.6526		

The average values of the number of seedlings that died and the leaf area destroyed in the whole experiment, irrespective of varieties and treatments, during the four seasons in which this experiment was conducted, together with all possible differences are shown in Table XX.

TABLE XX

Mean differences between seasons

Death of seedlings						Leaf area destroyed					
Season	1930-31	1931-32	1932-33	1933-34	Mean value	Season	1930-31	1931-32	1932-33	1933-34	Mean value
1930-31	...	**23·93**	—12·02	—8·86	24·51	1930-31	...	**3·25**	—1·83	**7·78**	4·43
1931-32	—23·93	...	—35·95	—32·79	48·44	1931-32	—3·25	...	—5·08	**4·53**	7·68
1932-33	**12·02**	**35·95**	...	**3·16**	12·49	1932-33	**1·83**	**5·08**	...	9·61	2·60
1933-34	**8·86**	**32·79**	—3·16	...	15·65	1933-34	—7·78	—4·53	—9·61	...	12·21

Critical difference at one per cent = 1·698 Critical difference at one per cent = 0·310

It is evident that in the four barley types under study *H. sativum* brought about 12·49 per cent deaths in the seedling stage in 1932-33, 15·65 per cent in 1933-34, 24·51 per cent in 1930-31 and 48·44 per cent in 1931-32. The destruction of leaf area was least in 1932-33 working out to be only 2·60 per cent and maximum in 1933-34 when it was 12·21 per cent. In 1930-31 it was 4·43 per cent, while in 1931-32 it was 7·68 per cent. The positive differences which are statistically significant are in thick type in the above table.

The behaviour of these four types of barley during the seasons under review may be gauged from the table which follows :—

TABLE XXI

Mean differences between varieties of barley

Death of seedlings						Leaf area destroyed					
Varieties	T. 7	T. 12	T. 17	T. 21	Mean value	Varieties	T. 7	T. 12	T. 17	T. 21	Mean value
T. 7 .	...	—1·67	**6·21**	—0·61	24·29	T. 7 .	...	—4·24	—8·42	—7·54	11·78
T. 12 .	*1·67*	..	**7·88**	1·06	22·62	T. 12 .	**4·24**	...	—4·18	—3·30	7·54
T. 17 .	—6·21	—7·88	...	—6·82	30·50	T. 17 .	**8·42**	**4·18**	..	**0·88**	3·36
T. 21 .	0·61	—1·06	**6·82**	...	23·68	T. 21 .	**7·54**	**3·30**	—0·88	...	4·24

Critical difference at one per cent = 1·698
Critical difference at 5 per cent = 1·292

Critical difference at one per cent = 0·310
Critical difference at 5 per cent = 0·236

Type 17 seems to show maximum and type 12 the minimum percentage of deaths during the seedling stage, whereas type 7 shows the maximum and type 17 the minimum destruction of leaf area due to the fungus.

Similarly, the differences of mean values due to the various seed treatments are presented in Table XXII below :—

TABLE XXII

Mean differences between different seed treatments

Treatments	Death of seedlings					Treatments	Leaf area destroyed				
	A	B	C	D	Mean value		A	B	C	D	Mean value
A .	...	—1·52	—1·39	—8·97	28·24	A . .	..	0·48	0·73	—0·02	6·43
B .	*1·52*	...	0·13	—7·45	26·72	B . .	—0·48	...	*0·25*	—0·50	6·91
C .	*1 39*	—0·13	...	—7·58	26·85	C . .	—0·73	—0·25	...	0·75	7·16
D .	8·97	7·45	7·58	...	19·27	D . .	0·02	0·50	0·75	...	6·41

Critical difference at one per cent = 1·698
Critical difference at 5 per cent = 1·292

Critical difference at one per cent = 0·310
Critical difference at 5 per cent = 0·236

The results are too obvious to need further comments and it is apparent that seed disinfection reduced death during the seedling stage but had no appreciable effect in reducing the secondary attack.

X. CONCLUSION

H. sativum causes considerable damage to the barley crop in Pusa every year. The amount of infection varies with locality, year and the variety of barley. All Pusa types of barley are more or less susceptible to the disease. It has been found that certain ecological factors, especially temperature, play an important part in varying the severity of the disease. *H. teres* also has recently been introduced at Pusa but is restricted to certain types only, whereas *H. gramineum* is either absent or very rare and occurs only during certain years when a foreign type is grown at the Pusa Farm. This is perhaps due to the fact that the range of temperature at Pusa is not suitable for the growth of *H. gramineum*. According to Johnson [1925] low soil temperature (10° to 12°C.) favours infection of barley and very little stripe disease occurs at soil temperatures higher than 20° C. This low temperature does not occur at Pusa at the time of sowing or harvesting and this may account for the absence of the disease. In the United Provinces and also in Nepal, where the soil temperature is lower, this disease makes its appearance. That high temperatures are favourable for the development and the spread of the disease caused by *H. sativum* has also been noticed by Dosdall [1923], McKenny [1923] and Henry [1924].

Predisposition plays an extremely important part in the degree of infection, *e.g.*, type 21, which is less susceptible, was rather severely attacked during 1933-34. This may be due to the conditions unfavourable for the growth of the barley plant but favourable for the growth of the fungus. Thus, when a resistant type is confronted by unfavourable conditions, it also becomes liable to infection.

The reaction of the soil also determines to a great extent the degree of infection, and this perhaps is the reason why infection is more in certain fields and less in others or is greater in one part of the field and milder in another part of the same field.

Weather conditions at harvest time or just before it, exert an influence on the percentage of infection in the ear and grain. Dry weather at the critical period tends to reduce the intensity of contamination while a wet period favours the development of *H. sativum*.

Various fungicides tried during the four years under review helped to check the disease to a certain extent. The superiority of uspulun in controlling the disease was confirmed several times during 1931-32, 1932-33 and 1933-34 and ceresan which was tried during 1933-34 proved to be still more effective. Though these fungicides controlled the disease to a greater or lesser extent during the seedling stage they usually failed to check the attack on the leaf surface owing to secondary infection taking place by means of air-borne spores from different fields of barley, wheat and other wild grasses on which *H. sativum* thrives. Further, *H. sativum* is capable of living saprophytically in the soil and can infect treated seed. It has been noticed that the plots on which barley is grown year after year are the worst affected both during the seedling and the mature stages of the plant.

It is not sufficient only to disinfect barley seed one year and save the grain from the resultant crop for sowing in the succeeding year, as such grain may be heavily contaminated with spores and mycelium of *H. sativum* due to secondary infection. It is essential that seed should be treated every year with an effective disinfectant of the ceresan type, if the disease is to be controlled in the primary phase. As far as the obvious loss is concerned, the primary phase (foot-root and root-rot) is of course the more important than the secondary infection on account of the greater mortality during the seedling stage.

Since the disease, especially the secondary infection, cannot effectively be controlled by seed treatment alone, the necessity for evolving new types resistant to the disease becomes apparent. It has been observed that there is a differential varietal resistance in the Pusa types of barley, that is, some are susceptible while others are resistant to a certain degree. The use of existing resistant varieties and breeding of new types with more suitable agronomic characters is therefore one of the most promising methods of preventing *Helminthosporium* disease. This method is receiving the attention it deserves at Pusa. In addition to breeding resistant varieties, it is advisable to plant clean seed and to have crop rotation in order to reduce the possibility of infection from the soil.

XI. Summary

1. Three species of *Helminthosporium* occur on barley in India, *viz.*, *H. sativum* P. K. & B., *H. teres* Sacc. and *H. gramineum* Rabh.

2. *H. sativum* is common at Pusa and in its neighbourhood. It is responsible for foot-rot and root-rot, head blight and spot formation on all aerial parts. It lowers the percentage of seed germination and reduces the crop yield.

3. *H. teres* also occurs at Pusa but is restricted to such types as have been introduced from outside. It is altogether absent on the types of barley grown locally. *H. gramineum* is very rare at Pusa.

4. The amount of damage done by *H. sativum* to various types of Pusa barley, varies from type to type, from season to season, and from plot to plot. In some years it occurs in a virulent form, *e.g.*, during 1934.

5. The percentage of leaf surface destroyed by *H. sativum* and *H. teres* during 1930-1934 on various types of barley is given.

6. Various fungicides were used to control the disease, *i.e.*, sulphur, formalin, uspulun, mercuric chloride and ceresan. Mercuric compounds were the most successful but none of the disinfectants was found to check the disease entirely. This is due to the fact that the organism lives in the soil and is parasitic on several grasses and also on wheat. Thus, repeated infections may occur during the growing season and seed treatment may not eliminate the disease altogether. Suitable treatment of each year's seed will, however, check the disease during the more important primary phase.

7. Differences exist in the susceptibility of different varieties to the attack of *H. sativum* but the degree of infection is influenced by environmental factors such as temperature, humidity, etc.

8. Since the disease, especially the secondary infection, cannot be effectively controlled by seed treatment alone, the study of varietal resistance is of great importance.

The writers are very grateful to Mr. Azmatullah Khan, Fieldman, Mycological Section, Pusa, for the ungrudging help rendered in making observations of various field experiments during the course of this study.

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SEEDLING BLIGHT OF *CINCHONA LEDGERIANA* MOENS CAUSED BY *PHYTOPHTHORA PALMIVORA* BUTL. IN THE DARJEELING DISTRICT

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(Received for publication on 25th February 1935)

(With Plate XX)

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I. Introduction

Cultivation of cinchona was taken up in India on or about 1866 at which time Sir Clements R. Markham brought the seed of *Cinchona officinalis* L. and *C. Calisaya* Weddel from Bolivia and Peru. The first plantation was laid down in the Nilgiri Hills of the Madras Presidency and since then its cultivation has been extensively taken up in the Sikkim plantations of the Darjeeling district and in

certain parts of Burma. The cinchona plant does not grow below an altitude of 2,000 feet. The crop needs copious rainfall, a cool and humid climate and at the same time well-drained land, as water-logging is fatal to its growth. In the Darjeeling district it is grown on steep mountain sides and all attempts to grow these plants in the plains were unsuccessful.

A serious seedling-blight disease was first observed at Mungpoo, in the Darjeeling district in 1928 in seed-beds and, as it was causing a good deal of loss to the plantation, the investigation was taken up.

II. Symptoms

The disease manifests itself for the first time at the collar of an affected seedling where the discoloration of affected tissues becomes evident. The discoloured area gradually extends above till it reaches the cotyledons which then become limp and bend over. The leaves gradually become yellow, curl inwards, and in severe cases are shed, but this is not so very common. The discoloration is followed by rotting and when the decomposition of the bark tissues has started and the inner vascular regions are affected the seedlings hang down from the affected portion and die.

When the weather is moist, copious aerial mycelium can be seen on the affected seedling and microscopic examination reveals the presence of numerous sporangia attached to broad unseptate mycelium indicating that the pathogene is a Phycomycete. The mycelium is broad, with a granular protoplasm, and penetrates the bark, cortex and the wood. It is found both in the cells and between the cells. When the mycelium penetrates the cell wall, it becomes constricted just at the point of perforation. Haustoria are not observed. The cell contents are destroyed and in advanced cases the walls collapse. The sporangia are rather loosely attached to the stalks and at the slightest puff of wind they are blown to other plants that are near, and the disease may spread in this manner also. Several seedlings at Mungpoo were found to die from the top downwards, though they were affected by the same Phycomycete, indicating that the infection can also be aerial, that is, from the sporangia that are blown about from the affected seedlings or by zoospores which may also be splashed by rain on to the leaves and may bring about aerial infection. A close search for oospores on diseased seedlings was made but none were found.

III. Isolation

Pieces of affected seedlings were first surface-sterilised by washing them in 0·1 per cent corrosive sublimate solution and then in several changes of sterile water. They were then incubated in moist chambers. A copious growth of mycelium was found on the incubated tissues in about five days time ; the mycelium was profusely sporulating. Bits of this mycelium were transferred to Quaker oat agar tubes and culture of the fungus was thus obtained. Single sporangial cultures were then obtained by using Keitt's [1915] plating method. The fungus isolated was a species of *Phytophthora*.

A seedling of *Cinchona ledgeriana* infected with *Phytophthora palmivora* Butl. (natural size)

IV. Pathogenicity

Inoculation experiments with *Phytophthora* isolations were carried out at Pusa and at Munsong and Sureil near Mungpoo in the Darjeeling district. For this purpose, seedlings of cinchona that were about one foot high were selected and brought down to Pusa for inoculation purposes. The inoculum was placed either on the base of the stem or on the tender leaves.

After placing the infective material in position, it was covered with wet cotton wool and the cotton was kept constantly moist. The results of the test are recorded in Table I.

Table I

Results of inoculating cinchona seedlings with a species of Phytophthora

No. of plants inoculated	No. of plants taking infection	Position where inoculum was placed
15	9	Leaves
15	15	,,
15	10	,,
7	7	Collar
10	6	,,

In each case five plants were kept as controls. The symptoms on the inoculated plants (Plate XX) were similar to those noticed on naturally infected plants. The controls received the same treatment as the infected plants except that the infective material was not placed on them. All of them remained quite healthy during the course of the experiments and much later. From the infected plants the causal organism was isolated and it agreed with the original fungus. The pathogenicity of *Phytophthora* sp. isolated from the diseased seedlings was thus established. At the time the inoculations were done at Pusa the temperature was in the neighbourhood of 27°C. and the humidity was very high. At Mungsong where the right conditions for getting successful inoculations were available, infection experiments carried out also proved the pathogenicity of the fungus.

V. Morphology

The morphological features of the organism were studied on Quaker oat agar, a few other *Phytophthora* spp. being also included in this study for purposes of comparison, *viz.*, *P. palmivora* Butl., *P. meadii* McRae, *P. colocasiae* Rac., and *P. parasitica* Dast. It was noted that while these species put forth profuse aerial

growth, the cinchona *Phytophthora* was less profuse. Macroscopically it appeared to bear resemblance to *P. palmivora* and to a less extent to *P. meadii* than to the other fungi included in the study. The aerial hyphae appeared to be rather broader than the submerged hyphae, the hyphae were unseptate when young but put forth thin septa at the time of forming the fruit bodies. The mycelium was richly granular and became knotted in some cases. In cultures the sporangia were formed rather sparingly. They were narrowly and in some few cases broadly ovate, pear-shaped, ellipsoidal or sometimes almost spherical. The papillae were very prominent. The sporangium bore at the basal end a distinct pedicel. They were mostly borne terminally but a few were intercalary. For obtaining a large number of sporangia, a bit of the vigorously growing mycelium was transferred to plain, sterile water and incubated overnight at 20°C. Sporangia thus formed were smaller in size than those obtained in culture, which might possibly be due to lack of food material.

Germination of the sporangia may be direct or indirect. In the former case slightly higher temperatures were observed to be necessary but indirect germination by means of zoospores through the papillate end of a sporangium was favoured by low temperatures. Each sporangium may put forth zoospores which swim for a while, become round and then come to rest ; the number of zoospores varied from 13 to 29 according to the size of the sporangia. In the case of direct germination by means of germ tubes, secondary sporangia may be formed which produce the zoospores. Two hundred sporangia were measured from a water culture at 25°C. and the range for length and breadth was 17·5—58·5μ and 9·0—30·0μ, mean being 33·3 × 17·6μ. In old cultures chlamydospores predominate and measurement of 200 of them indicated that they had an average diameter of 37·3 μ. A statistical analysis of the spore measurements is given later. Oospores were found neither in the host tissues nor in artificial cultures.

VI. Influence of environment on spore production

(*a*) *Humidity*

Slant cultures on Quaker oat agar were kept in a desiccator for comparison with similar slants at laboratory humidity. The *Phytophthora* grown in a dry atmosphere showed retardation of growth and enhanced production of chlamydospores.

(*b*) *Light*

Mycelium was placed in distilled water in watch glasses of which six were placed in bright light against a north window and another set of six in a dark chamber, and was examined at the end of every hour. It was observed that the sporangia that were formed germinated by means of zoospores in the light, but that only direct germination took place in the dark. Light therefore seemed to favour indirect germination through the agency of zoospores. Few chlamydospores and no oospores were observed.

VII. Mixed cultures

As the fungus did not form oospores in cultures, attempts were made to induce oospore formation by growing this *Phytophthora* together with others in the same Petri dishes. First attempts to induce oospore formation in this manner were made by Clinton [1909-10] and since then Gadd [1924]. Lester-Smith [1927], Ashby [1929, 1 ; 1929, 2] and Narashimhan [1930] have succeeded in obtaining oospores by growing various strains in mixed cultures. In the present study, investigation was carried along two lines : (1) paired cultures by growing together various isolates, all of which were monosporangial in origin, from diseased cinchona plants made on various occasions, to see if heterothallism existed : (2) paired cultures using one of the following fungi against one of the cinchona isolates :—*P. colocasiae*, *P. parasitica* from guava, *P. parasitica* from cotton, *P. meadii*, *P. faberi* and *P. palmivora*. In paired cultures using various combinations of the isolates from cinchona seedlings, no oospores were at any time found, indicating that there may not be heterothallism in this fungus at least so far as the isolates that were used in the present study are concerned. In the case of paired cultures using the other *Phytophthora* spp. oospores were obtained in all cases except *P. meadii*. The results are tabulated in Table II, together with extreme and mean measurements.

Table II

Oospore formation in paired cultures of Phytophthora *on Quaker oat agar in Petri dishes*

Nature of isolates grown together	Oospore formation	Measurements of 100 oospores	
		Range in μ	Mean in μ
Cinchona P1 × Cinchona P2 . . .	—	..	..
Cinchona P *X P. colocasiae* . . .	+	23·4—39·6	31·5
Cinchona P *X P. parasitica* (guava) .	+	21·6—34·2	28·2
Cinchona P *X P. parasitica* (cotton). .	+	25·3—36	30·4
Cinchona P *X P. meadii* . . .	—	..	..
Cinchona P *X P. faberi* (cocoa) . .	+	23·4—36	29·9
Cinchona P *X P. palmivora* (palm) . .	+	23·4—34·2	29·4*

*Only fifty oospores were available for measurement in this case.

The Petri dishes containing the paired cultures were placed in an incubator registering 22°C. Oospore formation took place in all cases at the place where the mycelium of the two isolates met. It may be mentioned that at ordinary laboratory temperatures, around 28°C., no oospore formation took place.

At the junction of the mycelium from the two fungi, there was usually a pale yellow line and on microscopic examination, it was found that oospore formation at this place was quite profuse. The antheridia were amphigynous, the oogonial wall was smooth, thin and hyaline while the wall of the oospore was rather thick and yellowish. The oospores looked rather yellowish in colour. Attempts to germinate the oospores were negative.

The question arises whether the oospores produced in these mixed cultures were the result of hybridisation or of some stimulus which one fungus provided to the other for their development. Literature on this point has been recently summarised by Tucker [1931], and Narashimhan's [1930] view, that there is heterothallism in the species of *Phytophthora* with which he worked and that the oospores are produced as a result of hybridisation between the + and — strains does not seem to find favour. In the present studies the isolates did not form oospores in pure culture nor in mixed cultures of cinchona strains but only in mixed cultures with different species of *Phytophthora*. The trials are too few, however, to enable one to draw any definite conclusions from these data, but the fact that the oospores were formed only at the junction of the mycelia from the two different cultures, is significant. It may mean either that the oospores are a result of hybridisation or that some stimulus is provided by one culture to the other for oospore formation, which need not be necessarily chemotropic but can also be thigomotropic.

VIII. Identity of the Cinchona *Phytophthora*

Finally the question of the identity of the *Phytophthora* isolated from the diseased seedlings of cinchona remains to be determined. For this purpose several criteria that are usually applied for the determination of species of *Phytophthora* were applied and the results are presented below. But it has to be emphasized here that the question of taxonomy of the species belonging to this genus is extremely difficult. Attempts by Rosenbaum [1917] to monograph the genus and those by Leonian [1925] to classify the species on the basis of physiological responses have not been conclusive. The recent effort of Tucker [1931] has however clarified the ideas regarding the taxonomy of the genus to a considerable extent.

The following criteria for the determination of the specific rank of cinchona *Phytophthora* were tried :—

(*a*) Pathogenicity and cross inoculation experiments.

(*b*) Critical temperature for growth.

(*c*) Ability to infect certain selected hosts.

(*d*) Morphological comparison including measurement of sporangia.

(*a*) *Pathogenicity tests.*—Pathogenicity tests were conducted by testing the parasitism of three species of *Phytophthora* on cinchona seedlings, and by testing the parasitism of the species from cinchona on castor, palm, betel vine and colocasia. Healthy and vigorously growing seedlings of cinchona were obtained for this purpose and they were carefully infected with the respective species using the usual aseptic methods. The results of these tests are given in Table III.

TABLE III

Pathogenicity of certain Phytophthora *spp. to cinchona seedlings*

Name of parasite	No. of cinchona seedlings	No. killed
P. parasitica	14	*Nil*
P. colocasiae	15	,,
P. palmivora	15	7
Cinchona P.	15	15

From the data recorded in Table III, it will be noted that *P. parasitica* and *P. colocasiae* are unable to parasitize cinchona seedlings but that *P. palmivora* can do so. Of the fifteen plants that were infected with this fungus seven died showing the typical symptoms while all the plants infected by the cinchona fungus succumbed within that time. These infection experiments indicate that the cinchona species may be a strain of *P. palmivora.* This point was settled by the following further experiments.

(*b*) *Critical temperature for growth.*—Tucker [1931] considers that the maximum temperature at which the growth of a *Phytophthora* can take place may yield a clue as to its identity. While this is not an absolute criterion, it certainly helps in arriving at a conclusion along with the other criteria. The fungus was grown in Petri dishes on corn-meal agar and placed in incubators that were accurately adjusted to 32·5°, 35° and 37·5°C. None of the species included in this test grew at 37·5°C. At 35°C. *P. parasitica, P. colocasiae* and *P. palmivora* grew slightly. The cinchona *Phytophthora* grew only at 32·5° and not at the higher temperature.

That the cinchona *Phytophthora* did not grow at 35°C. when *P. palmivora* did, is possibly due to the fact that while *P. palmivora* was an isolate from a palm tree from the plains, the cinchona *Phytophthora* came from the mountain valleys where it may have, in the course of years, become accustomed to a cooler temperature. Attention may be drawn in this connection to the twenty-two isolations of *P. palmivora* studied by Tucker [1931]. While he considers them all to belong to the same species, they show considerable variation in their response to the same temperature so that the slight variation seen between the cinchona *Phytophthora* and *P. palmivora* in this case is not very material.

(*c*) *Ability to infect certain hosts.*—In order to ascertain the ability of cinchona *Phytophthora* to infect certain hosts, its parasitism was tried on leaves of castor, colocasia, betel vine and palm. The usual aseptic precautions were taken in these tests and the standard methods of testing were tried. The results are given below:

TABLE IV

Parasitism of cinchona Phytophthora *on certain hosts*

Host	No. infected	No. that took infection
Castor leaves	17	12
Colocasia leaves	12	*Nil*
Betel vine leaves	14	,,
Palm leaves	15	8

The results show that only castor leaves and palm leaves are infected by the cinchona *Phytophthora*. Even though the castor leaves are attacked by the cinchona fungus, the castor fungus, *P. parasitica*, is unable to infect cinchona seedlings. In the case of infection of palm leaves, however, the cinchona fungus is not only able to infect the palm leaves but the palm *Phytophthora* is able to infect cinchona seedlings. It would appear therefore that the *Phytophthora* from cinchona is not *P. parasitica* but a strain of *P. palmivora*.

(*d*) *Morphological comparison and spore measurements.*—The above conclusion was further supported by the morphological features of the two fungi and their spore measurements. Butler [1910] mentions that the sporangia of *P. palmivora* have a distinct stalk at the base. The sporangia of cinchona *Phytophthora* possess also a distinct stalk. It therefore resembles *P. palmivora* in this respect rather than *P. parasitica* which according to McRae [1934] is without stalks. Furthermore according to Butler the papilla of *P. palmivora* are very prominent. Critical comparison of the papilla of cinchona *Phytophthora* again showed its close resemblance to *P. palmivora* rather than to *P. parasitica*.

For the purpose of comparing the spore sizes, 200 sporangia were measured, both of cinchona *Phytophthora* and *P. palmivora*. The method of obtaining these sporangia is already given in a previous section. The measurements were arranged in the form of frequency arrays, both for length and breadth, and these are given in Table V, together with the mean measurements. For seeing whether there was any agreement between the lengths and breadths of *P. palmivora* and cinchona *Phytophthora*, Pearsons' χ^2 test as applied by Mundkur [1934] was used. The values of χ and P obtained in each case are also given at the end of Table V.

TABLE V

Frequency tables of length and breadth of the sporangia of cinchona Pnytophthora *and* P. palmivora

Mid-point	Cinchona *Phytophthora*		*P. palmivora*	
	Length in μ	Breadth in μ	Length in μ	Breadth in μ
9·5		18		15
13·5		50		35
17·5	1	76	1	56
21·5	20	39	13	60
25·5	36	14	29	27
29·5	28	3	27	6
33·5	42		41	1
37·5	28		35	
41·5	24		29	
45·5	13		11	
49·5	4		10	
53·5	2		1	
57·5	2		3	

Mean length 33·3±·39. Mean length 34·7±·36.
Mean breadth 17·6±·19. Mean breadth 18·9±·23.

Agreement between lengths of sporangia of cinchona *Phytophthora* and *P. palmivora* } $\chi^2 = 6 \cdot 78$.

P for $n = 11$ is between ·80 and ·70 (Fisher's table)

Agreement between breadths of sporangia of cinchona *Phytophthora* and *P. palmivora* } $\chi^2 = 43 \cdot 79$.

$P < 0 \cdot 01$

It will be noted from the data recorded in Table V that the sporangia of cinchona *Phytophthora* have a slightly smaller mean than those of *P. palmivora* but the mean difference is not however significant as the value of P is high, between ·80 and ·70. With respect of length therefore the two fungi can be considered as same.

With respect to breadth, the sporangia of cinchona *Phytophthora* are slightly narrower than those of *P. palmivora*. The mean difference in this case, however, is significant as the value of *P* is less than ·01. Comparison with the measurement of the sporangia of *P. colocasiae*, and *P. parasitica*, made in connection with some other investigations at the same time as these investigations, showed however that the cinchona fungus agreed closely in respect of sporangial size with *P. palmivora* rather than with the others.

In a recent paper Celino [1934] has described a *Phytophthora* on cinchona from the Philippines and named it *P. faberi* Maubl. When discussing the taxonomy of cinchona *Phytophthora* he states " According to Rosenbaum's tentative table for the separation of the species of the genus *Phytophthora* the absence of oospore places the fungus in the *Faberi* group. The Philippine *Phytophthora*, cinchona strain therefore falls under this category ". Reinking [1923] also considered his isolations of *P. faberi* distinct from *P. palmivora* because they did not produce oospores. It is customary to consider isolations from cacao, *P. faberi*, and those from palms, *P. palmivora*, but it is doubtful whether any real difference exists between the two species. Celino [1934] has mainly depended on the size of sporangia and chlamydospores for the classification of his species. Tucker [1931], Leonian [1925] and other workers have shown that the sporangia and chlamydospores of *Phytophthora* of the same species vary so much in size that they cannot be depended upon for specific identification. Butler [1924] and Gadd [1924] considered *P. faberi* and *P. palmivora* morphologically similar, while Leonian [1925] considered them alike physiologically. Lester-Smith [1927], Seal [1928], Ashby [1929], Thomson [1929] and other writers regarded the two species as identical and the majority recognised the validity of *P. palmivora* as the specific name. The writer therefore prefers to name his isolation from cinchona as *P. palmivora* Butl.

IX. Summary

1. A species of *Phytophthora* causes a seedling blight of cinchona in the Sikkim plantation of the Darjeeling district.

2. Inoculation experiments on the stems and leaves of cinchona with the isolates proved the pathogenicity.

3. The optimum temperature for the growth of cinchona *Phytophthora* is found to be about 24°C. Above and below this temperature the growth decreases and at 35°C. there is no growth at all.

4. Cross-inoculations with cinchona *Phytophthora* and *P. palmivora* Butl. on cinchona and palm leaves respectively gave successful results.

5. Morphological comparisons of cinchona *Phytophthora* with *P. palmivora* Butl., *P. meadii* McRae, *P. colocasiae* Rac. and *P. parasitica* Dast. indicated that it agrees most closely with *P. palmivora* Butl.

6. Statistical comparison of the sizes of sporangia of cinchona *Phytophthora* and *P. palmivora* Butl. were made and it was found that in length the sporangia of both the fungi agree but in breadth the sporangia of cinchona *Phytophthora* are slightly narrower than those of *P. palmivora* Butl.

7. It is concluded therefore that the cinchona fungus is a strain of *P. palmivora* Butl.

X. Acknowledgments

The writer wishes to take this opportunity to render thanks to Dr. W. McRae for suggesting the problem and for providing the necessary facilities for conducting the research. Grateful acknowledgments are made of the help given by Dr. B. B. Mundkur in preparing the paper and in aiding in the biometrical analysis, and to Dr. Mitra for making useful suggestions.

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STUDIES ON THE ROOT-ROT DISEASE OF COTTON IN THE PUNJAB

I. SYMPTOMS, INCIDENCE AND CAUSE OF THE DISEASE

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(Received for publication on 5th March 1935)

(With Plates XXI and XXII and two text-figures.)

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I. Introductory

The root-rot disease of cotton is very serious in the Punjab and causes heavy damage to the crop every year. It has been prevalent for many years and affects both American and indigenous (*desi*) cottons. The disease attacks the roots exclusively and brings about at first wilting of shoots and then death of the entire plant. The damage caused by the disease to cotton has been found to vary a great deal in different fields. Observations made on the cotton crop in the Sargodha Colony showed that the percentage of damage was 1-15 in different fields; while that on the Lyallpur Agricultural Farm, on the average, the loss has been about 3·3 per cent. In certain individual fields the loss is very great. In some plots of the Botanical area at Lyallpur, about 11 per cent of the

crop was destroyed by the root-rot disease annually. In other plots, however, as much as 30 per cent plants were killed by this disease. In certain fields on the Agricultural Farm, Montgomery, over 50 per cent of the crop had been reported as affected by root-rot.

Incidence of the disease in zamindars' area.—Reports received regarding the occurrence of this disease in zamindar's fields in various parts of the Province have shown that the disease occurs commonly wherever cotton is grown. In some cases, the damage reported has been as great as 60 per cent of the whole crop. Taking into consideration the extent of the disease for the whole province, the average loss, at a moderate estimate may be put at about 2·5 per cent. On this basis the area of the cotton crop damaged by the disease would be 56,250 acres, taking $2\frac{1}{4}$ million acres as the total area under cotton in the Province. If four maunds be taken as the average outturn per acre and Rs. 7 as the price of cotton per maund, the total value of the cotton crop destroyed by the disease would amount to (56,250 acres × 28) = Rs. 15,75,000 ($15\frac{3}{4}$ lakhs) *. This is evidently a very heavy loss. But if the loss of individual fields is calculated, it would come to an alarming figure.

An investigation of the cotton root-rot disease had been carried on by the Punjab Agricultural Department for some time. Preliminary observations made on the diseased roots showed the presence of fungal hyphae and spores and pointed to the occurrence of soil fungi of the order of *Fusarium*. But nothing definite was established regarding the causal organisms. The problem remained unsolved and no further light had since been thrown on the presence of any effective parasitic fungi, as the inoculation of healthy plants failed to produce the disease. The investigation of the problem has been taken in hand under a scheme of the Indian Central Cotton Committee since 1932. The work so far done is briefly described in the paper.

II. Symptoms of the disease

The first and prominent symptom of the disease is the complete wilting of the plant. Early in the morning one can see plants, which have suddenly and completely wilted. From top to bottom every leaf droops down and it is a matter for surprise that plants looking perfectly healthy are killed outright within a day. Sometimes a wilted plant may recover during a cool night, in wet weather or by irrigation, but this is very rare. Usually when once a plant wilts, there is no recovery.

The suddenness of wilting is a characteristic feature of this disease.

Plants affected by root-rot can be easily pulled out of the ground. By removing the soil carefully around the roots of freshly attacked plants, it has been observed that except the tap root and a few secondary roots which hold

* This is a rough estimate and actual survey needs to be made and will be carried out as soon as an opportunity arises.

the plants in the soil, all other roots are decayed and detached. The disease actually starts much earlier and the above-ground manifestation of the disease in the form of wilting is, therefore, a very late symptom. The tip of the root of a freshly wilted plant is slightly moist and sticky to the touch. On a white surface it leaves a yellowish stain. The bark tissue is much shredded. On some freshly wilted plants the yellowing and shredding extends above the terminal portion. Plants that have recently succumbed to the disease have all their root tissues, particularly the bark, broken down into shreds. The central woody cylinder is not affected within but turns golden yellow in colour on the surface. In badly affected plants the wood also becomes brown or black. A plant showing typical symptoms of the root-rot disease is shown in Plate XXI, fig. 1. Plate XXI, fig. 2 shows the root-system of the same plant magnified.

Underneath the bark, there is a deposit of yellowish substance of granular nature. The lumen of the wood vessels is filled in several cases with yellow granular material. The shredded bark is found to contain fungal hyphae and these have sometimes been observed even in the xylem vessels.

Cotton is known to suffer from root-rot in the U. S. A. It is also reported from various other cotton-growing tracts of India. Although the Punjab root-rot of cotton resembles the Texas (U. S. A.) and the Gujerat (India) root-rot in some respects, it has distinct characters of its own. The suddenness of wilting, destruction of the major portion of the roots, yellowing of the tissues and shredding of the bark are common characters. When once a palnt wilts, it seldom recovers and the leaves of plants attacked by root-rot hang down over the branches and remain attached for some time. Peltier [1926] records that in the Texas root-rot, there is bronzing or yellowing of the leaves about 24 hours before wilting. This has not been observed in the Punjab. In Gujerat the sclerotia of *Rhizoctonia* are found in abundance within the bark and the fungus can be readily isolated [Likhite, 1934]. In the Punjab, sclerotia have been observed in rare cases.

In Texas the root-rot is caused by the fungus *Phymatotrichum omnivorum*, originally described by Shear [1907] as *Ozonium omnivorum*, but later assigned to the genus *Phymatotrichum* by Duggar [1916]. It makes its appearance when the squares are being formed and seldom earlier. The Gujerat root-rot is at its height during the flowering and boll developing stages. The Punjab root-rot appears when the plants are about two months old and so is much earlier and almost ceases during the flowering time. Taubenhaus and Killough [1923] mention that the strands of *Phymatotrichum omnivorum*, the causal organism of Texas root-rot are seen covering the epidermis. In the Punjab also fungal hyphae have in certain cases been seen on the surface of the roots. *Phymatotrichum omnivorum*, the Texas root-rot fungus, attacks a great variety of crops. It is still a matter for investigation whether Punjab root-rot fungus can infect other plants.

Fig. 1.
cotton plant affected with oot-rot.

Fig. 2.
Root system of the same plant.

Fig. 3. Cotton plants inoculated by transplanting method with the two species of *Rhizoctonia*, I and 22 (*a*). Control plants are also shown.

Fig. 4. Wilted cotton plants inoculated with *Rhizoctonia* species I and 22 (*a*) by root washing method along with controls.

III. Development of the disease

With a view to studying the cause of the disease, a number of cotton plants in rows showing different stages of root-rot symptoms along with apparently healthy plants were fastened together by means of cords. The ends of the cords were tied to vertical sticks at each end of the row. Trenches about 5 feet deep were dug on either side of each row and care was taken that none of the lateral roots were damaged. Then the soil was removed around the roots and most of the larger roots were thus exposed for examination in the 2nd week of August. As the plants were tied up, they did not fall over.

The results of the examination of roots thus exposed are given below. The plants showing the effect of root-rot at different stages are grouped separately :—

Group I. (*6 plants*).—All parts healthy ; tap root and lateral roots all sound. No fungal mycelium visible on the roots. In two plants, however, slight yellowing of the roots was observed.

Group II. (*2 plants*).—All shoots healthy ; tip of the tap root infected and rotting ; lateral roots healthy ; white mycelial, strands visible on several roots.

Group III. (*2 plants*).—All shoots healthy ; tap root rotten about 6-8 in. ; brown in colour ; soft ; bark shredding ; lateral roots slightly yellow.

Group IV. (*2 plants*).—Shoots wilted ; roots only slightly yellowish.

Group V. (*6 plants*).—Wilted for 2-3 days ; leaves drooping.

(*a*) (2 plants).—Tap root soft ; yellowish material exuding ; tap root infected ; lateral roots also slightly yellowish ; bark not peeling off.

(*b*) (2 plants).—Tap root infected ; brownish in colour about two feet above the tip ; yellowish material exuding ; tap root rotting and soft.

(*c*) (2 plants).—Tap root rotting ; brown and yellowish in places ; lateral roots showing symptoms of yellowing.

Group VI. (*8 plants*).—Wilted and dead for 7-10 days ; wilted leaves hanging on.

(*a*) (1 plant).—About 12 inches of tap root rotten ; bark shredding, yellow to dark brown in colour ; bark of the laterals peeling off and some rotting ; yellowish liquid exuding from large laterals.

(*b*) (2 plants).—Tap root infected and rotting over 6 inches length ; central cylinders brown ; bark around this point yellow on the inner side ; yellowish material exuding ; laterals yellowing and bark peeling.

(*c*) (3 plants).—Whole of tap root (27 inches) rotting and yellow; tip of the tap root brown and infected; lateral roots yellow and soft.

(*d*) (2 plants).—Tap root infected and brown; soft and rotting; laterals and tap root all showing yellowing and shredding.

IV. Incidence of the disease

To study the incidence and progress of the disease from week to week, certain plots in the Botanical area and Agricultural Farm at Lyallpur in which the disease is known to occur year after year were kept under observation for two seasons. The actual position of the infected plants was marked and the rate of progress of the disease was recorded. Similar observations were also made at Hansi and Montgomery.

Observations at Lyallpur indicate that the attack starts when the cotton plants are about six weeks old. The disease first appears in June. Within a fortnight the maximum percentage of attacked plants is reached. It, however, continues to be vigorous throughout July. In August the rate of death of plants gradually declines and the disease almost ceases by the end of September. Figs. 1 and 2 show the progress of the disease in a certain plot at Lyallpur during 1932 and 1933, respectively.

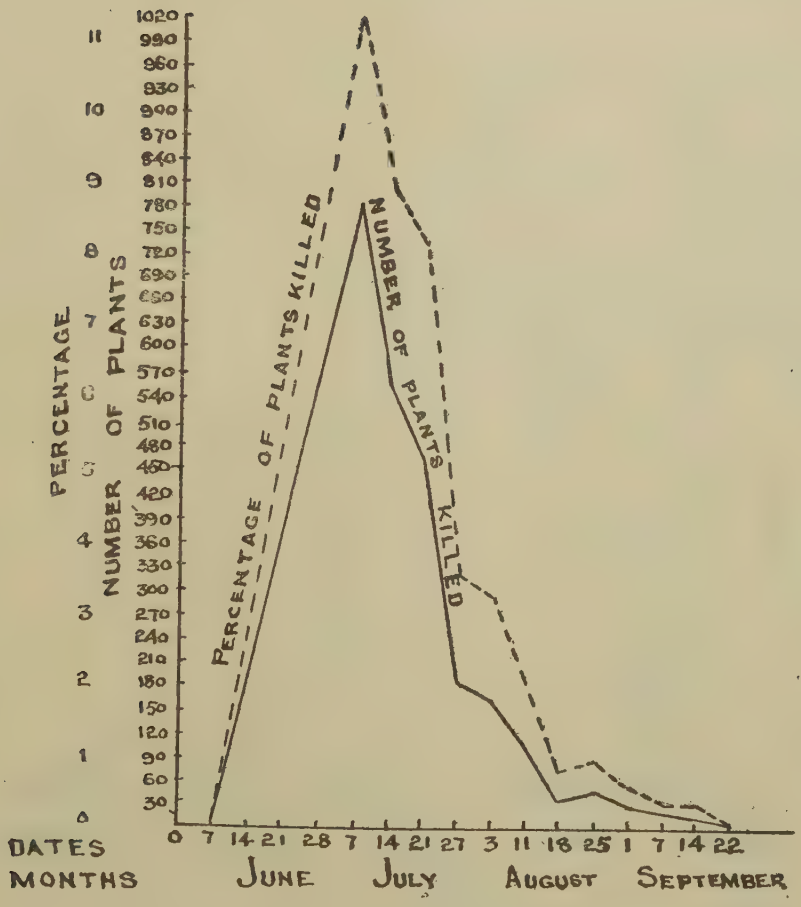

FIG. 1.

Square 46, plot 23

Mortality of plants due to root-rot, 1932

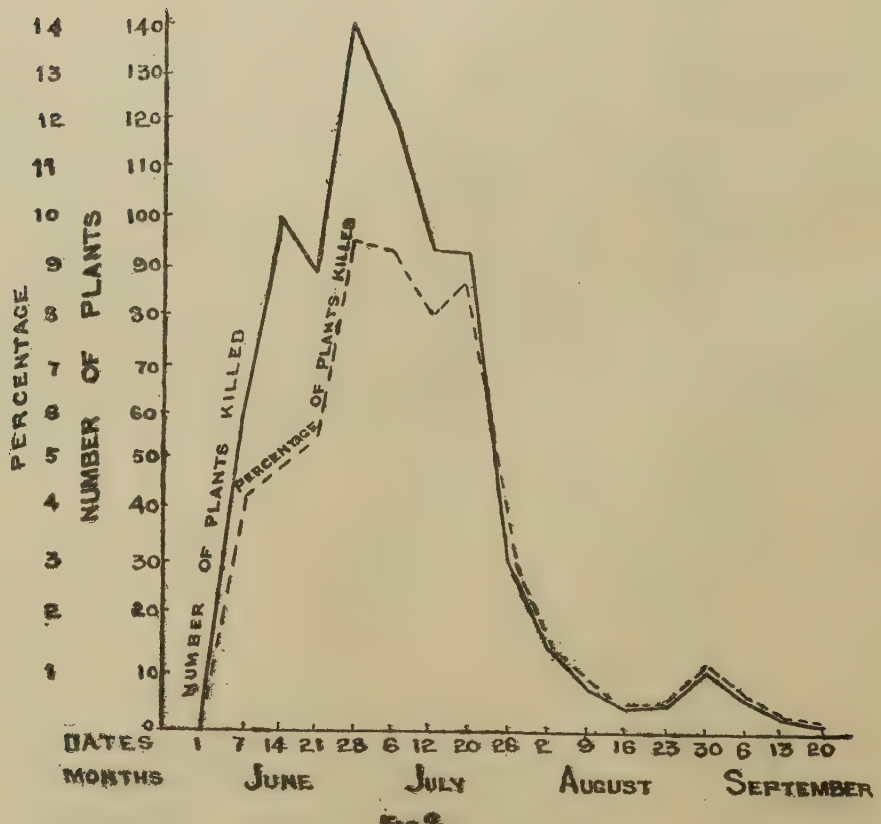

FIG. 2

Square 46, plot 23

Mortality of cotton plants due to root-rot, 1933

Similar observations were made at Hansi and Montgomery. The results obtained at Hansi were identical with those of Lyallpur. But at Montgomery the disease appeared a little later and the optimum attack was delayed till the first week of August. Table I shows the mortality due to root-rot in some severely affected plots under observation at Lyallpur and Montgomery in 1933.

TABLE I

Mortality due to root-rot at Lyallpur and Montgomery in some severely affected plots

Date of observation		Lyallpur			Montgomery		
Month	Week	Total No. of plants	No. of plants killed	Per cent attack	Total No. of plants	No. of plants killed	Per cent attack
June	1st	1,677	63	3·76	..	..	..
	2nd	1,614	102	6·31	..	..	..
	3rd	1,512	93	6·17	..	..	..
	4th	1,419	138	9·72	1,067	21	1·96
July	1st	1,281	120	9·37	1,046	79	7·55
	2nd	1,161	95	8·18	967	85	8·79
	3rd	1,066	93	8·73	882	89	10·09
	4th	973	35	3·60	793	89	11·22
August	1st	938	17	1·81	704	90	12·78
	2nd	921	9	0·97	614	65	10·59
	3rd	912	4	0·44	549	38	6·92
	4th	908	6	0·66	511	30	5·87
September	1st	902	13	1·44	481	16	3·33
	2nd	889	7	0·79	465	12	2·58
	3rd	882	2	0·23	453	8	1·77
	4th	880	0	0	445	6	1·35

It is clear from the above that in the plot under observation at Montgomery, out of the total of 1,067 plants at the beginning of the season, 632 succumbed to the disease showing a loss of 59·2 per cent. At Lyallpur the damage was 47·5 per cent. These two examples illustrate the ruinous damage in severely affected areas.

V. ISOLATION OF THE CAUSAL ORGANISM

In order to isolate causal organisms, material from different parts of the root system of the diseased plants was cultured. The plants that had wilted as a result of the disease were dug up and the earth washed off the roots. Small

pieces of tissues were dipped in mercuric chloride (1/1000) solution for 30-60 seconds. These were then given a thorough washing with sterile distilled water and transferred to Petri dishes containing plain agar. As soon as the mycelium grew from the transfers, inocula from these were planted on Richards' agar. For these isolations, diseased plants were obtained from various localities, *i.e.*, Lyallpur and neighbouring villages, Hansi, Sirsa, Montgomery, Sargodha and Khanewal. The total number of isolations from time to time went up to several thousands. The fungi thus reproduced were examined and it was observed that two species of *Rhizoctonia* appeared most frequently. For convenience these fungi will be referred to as I and 22 (*a*). In addition, two strains of a *Fusarium* appeared frequently, either alone or associated with *Rhizoctonia sp.* Besides *Rhizoctonia sp.* and *Fusarium*, a species of *Alternaria* and *Helminthosporium* appeared occasionally. Some saprophytic fungi were also found.

When the wilted plants were kept in a moist chamber overnight, a cottony white mycelium broke through the root tissue and appeared on the surface in abundance. This fungus was also found to be one of the above-mentioned *Rhizoctonia sp.*

It may be mentioned that Butler [1918] also isolated *Rhizoctonia* from roots of cotton plants attacked by root-rot in North-Western India.

VI. Identification of the fungi

Rhizoctonia sp. I is a strain of *Rhizoctonia solani* Kuhn group. It seems to differ from all other strains described so far.

Rhizoctonia sp. 22 (*a*).—This fungus is rather problematical. The scelrotial and hyphal characters are much like those of *Rhizoctonia bataticola* but no strain of it is known which produces so much white aerial mycelium. It is suggested that it may be called *Rhizoctonia bataticola* provisionally, as it is perhaps a saltant.

The two *Fusaria* (A and B) that were isolated have also been identified and are strains or 'Forms' of *Fusarium solani* (Mart.) App. and Wr. In the case of strain B, macroconidia are in definite sporodochia. Strain A does not produce macroconidia in definite sporodochia but is mixed with microconidia on the aerial mycelium. Moreover, macroconidia of strain A are broader than those of strain B.

VII. The causal organism

The various fungi isolated were tested for their parasitism on cotton plants of different ages growing in the fields as well as in pots under field and controlled laboratory conditions. Inoculations were made by various methods, which are discussed fully elsewhere. In Table II are given results of one of the typical sets of experiments carried out with plants in pots.

TABLE 11

Results of inoculations carried out in pots with different fungi

Cotton plant		Inoculated with *Rhizoctonia sp.* I			Inoculated with *Rhizoctonia sp.* 22 (*a*)			Inoculated with *Fusarium* A			Inoculated with *Fusarium* B		
Variety	Age (week)	No. inoculated	No. infected root-rot	Per cent infection	No. inoculated	No. infected root-rot	Per cent infection	No. inoculated	No. infected		No. inoculated	No. infected	
									Root-rot	Other causes		Root-rot	Other causes
4-F . .	3	11	1	9·09	12	11	91·66	12	0	0	10	0	0
Mollisoni .	4½	10	2	20·00	12	10	83·30	11	0	6	9	0	2
„ .	10	13	5	38·46	6	2	33·33	7	0	0	6	0	0
„ .	11	13	2	15·38	6	5	83·33	7	0	0	7	0	0
„ .	12	9	2	22·22	6	3	50·00	7	0	0	7	0	0

It is clear from Table II that *Rhizoctonia sp.* I and 22 (*a*) reproduce the disease. In the case of *Fusarium* A and B wilting of inoculated cotton plants once obtained was not typical of root-rot and other characteristic symptoms, *e.g.*, shredding and peeling off of root bark were also absent.

All the remaining fungi isolated were also included in the above pathogenicity tests, but they failed to produce infection or any symptoms of the disease, Throughout these experiments controls were kept side by side, *i.e.*, the plants were given exactly the same treatment as the ones inoculated, with the only exception that no fungal inoculum was added.

As the two species of *Rhizoctonia* appeared most frequently in isolations from diseased material and successfully reproduced the disease, these were selected for further study.

VIII. Material and methods

Only two varieties of cotton, namely, Desi *G. indicum* var. (*Mollisoni*) and American (4-F) were used for inoculation purposes throughout the investigation. The plants were raised in pots, containing sterilized soil, kept under normal field and controlled laboratory conditions. For field inoculation experiments, the crop was grown in rows 3 feet apart in plots, where root-rot disease had not appeared previously. During winter season the plants were grown in jars kept in hot water tanks maintained at the desired temperature, glass house and a special glass-walled chamber, which was fitted with an electric thermostat and heating bulb to provide the necessary temperature. During exceptionally cold nights the chamber was covered with felt blankets. The chamber was kept in a verandah where plenty of bright diffused light was available to the plants. For inoculation purposes both mass infection and single plant infection were tried. The source of inoculum was added either in the form of rots of freshly wilted plants due to root-rot, or pure cultures of the fungi, growing on sterilized* cotton roots, paddy or nutrient agar.

IX. Infection experiments

Preliminary infection experiments were carried on both in the field and in pots with all the fungi that had been isolated.

Two methods were employed for the infection experiments in the field.

(1) individual plant inoculation and

(2) mass inoculations.

Inoculations were made by direct infection (using the roots of diseased plants as the source of inocula) and pure culture infection methods.

*To obtain thorough sterilization it was found necessary both in the case of cotton roots and paddy to give three 20-minutes steam-sterilizations at 15 lbs. pressure on alternate days.

The roots of plants were exposed carefully to a depth of about eight inches and the inoculum was placed parallel to the tap root and in places in actual contact with laterals. Sterile moist cotton wool was applied with the inoculum to keep it moist. The soil was replaced and the plants were watered freely. Controls were treated similarly except that no inoculum was added.

In the preliminary inoculation experiments, tests were made with the different fungi isolated, but none of the plants developed any obvious signs of root-rot. In a few cases, however, where the plants were inoculated with either of the *Rhizoctonia sp.*, though they had perfectly healthy appearance, some of the roots were affected and showed characteristic shredding of the bark tissue. From such roots the *Rhizoctonia sp.* were readily isolated. These experiments were carried out late in the season and possibly the plants had outgrown the stage of infection.

Infection experiments were repeated with plants growing in pots using pure fungal cultures on nutrient agar as source of inoculum, but without success.

A number of further preliminary experiments were conducted, but the methods of inoculation were modified in a number of ways in order to eliminate certain factors which might be responsible for the failure of these infection trials. These experiments will now be described.

In the normal method of inoculations the fungal inoculum was introduced near the root and covered up with soil and watered. Under these conditions it was thought that accumulation of carbon dioxide or scarcity of oxygen might be the cause of failure to reproduce the disease. These factors were, however, ruled out by the observation that no attack took place when openings were made in the soil and the fungus was introduced close to the roots and those openings maintained and plugged with sterile cotton wool.

Transplanting method.—Cotton plants were grown in pots containing sterilized soil and when they were of the required age they were removed from these pots by washing out the soil with a fine but powerful jet of water, taking care so as not to injure the roots. These were then given a thorough washing in sterile distilled water and transplanted singly in glass jars containing sterile soil. The jars were fitted with corks having a hole (one cm. in diameter) in the centre and with one radial slit. The plants were put through these slits quite easily without injuring any of the roots. Sterile water was then added and the jars kept in a glass house during the summer and in hot water tanks (30-33° C.) during winter months. After about a week to ten days when the plants had established their roots, the soil from the top was removed carefully by means of a scalpel, inoculated with pure culture of the fungus on paddy and covered up with the soil that had been previously removed. Controls were treated similarly but in place of infected paddy, only sterilized paddy was introduced. The transplanted plants that did not look healthy were discarded before inoculation.

In another series of experiments the plants were transplanted into glass bottles that contained soil infected with cultures of different fungi on paddy.

Results of four inoculation experiments carried out by transplanting method are given in Table III. During all these experiments, plants of *Mollisoni* variety of cotton were used.

TABLE III

Results of inoculations carried out by transplanting method with different fungi

Date of		Age of plants in weeks	Inoculation with *Rhizoctonia sp.* I.			Inoculation with *Rhizoctonia sp.* 22 (*a*).			Inoculation with *Fusarium* A		Inoculation with *Fusarium* B	
Inoculation	Observation		No. inoculated	No. infected	Percentage infection	No. inoculated	No. infected	Percentage infection	No. inoculated	No. infected	No. inoculated	No. infected
28th Nov. 1932	5th Dec. 1932	8	4	4	100·0	4	2	50·0	4	0	4	0
10th Dec. 1932	15th Dec. 1932	10	6	3	50·0	4	0	0·0	5	0	4	0
5th April 1933	8th April 1933	6-7	5	5	100·0	5	3	60·0	4	0	3	0
21st April 1933	1st May 1933	7	4	4	100·0	3	2	66·6	2	0	3	0

Plate XXI, fig. 3 shows plants which had been transplanted and later inoculated with *Rhizoctonia sp.* I and 22(*a*). The inoculated plants showed wilting, whereas the checks looked healthy and remained sound.

The plants inoculated with the two *Rhizoctonia species* produced characteristic root-rot disease.

Inoculations with the two forms of *Fusarium solani* and species of *Helminthosporium* and *Alternaria* were unsuccessful. Controls always remained healthy.

In the first two experiments in Table III, the plants were transplanted and allowed to establish their roots and then inoculated. In the remaining two experiments the plants were transplanted directly into infected soil.

Root washing method.—In another series of experiments roots of the cotton plants of different ages growing in pots were exposed by washing off the soil around the roots up to a depth of about four inches. The fungus grown on paddy was then introduced, covered up with sterile soil and watered freely. Controls were treated similarly except that no inoculum was inserted. As far as possible aseptic conditions were maintained throughout the course of these experiments. Results of fourteen such inoculation experiments are given in Table IV.

TABLE IV

Results of inoculations carried out in pots by root washing method

Cotton plants		Inoculated with *Rhizoctonia sp.* I			Inoculated with *Rhizoctonia sp.* 22 (*a*)			Inoculated controls	
Variety	Age (weeks)	No. inoculated	No. infected	Per cent infection	No. inoculated	No. infected	Per cent infection	No. of controls	No. infected
Mollisoni	2	23	2	8·70	20	3	15·00	11	All healthy.
4-F	3	11	1	9·09	12	11	91·66	9	,,
4-F	3½	13	3	23·07	11	9	81·81	11	,,
Mollisoni	3½	14	11	78·57	15	5	33·33	12	,,
,,	4½	10	2	20·00	12	12	100·00	15	,,
4-F	5½	11	2	18·18	10	1	10·00	11	,,
Mollisoni	6	29	5	17·24	37	7	18·91	17	,,
,,	7	56	17	30·35	49	12	24·49	33	,,
,,	8	17	3	17·63	14	13	92·85	15	,,
,,	8	12	1	8·33	23	5	21·73	12	,,
,,	9½	15	3	20·00	15	5	33·33	13	,,
,,	10½	13	5	38·46	6	2	33·33	4	,,
,,	11	13	2	15·38	6	5	83·33	6	,,
,,	...	9	2	22·22	6	3	50·00	6	,,

Plate XXI, fig. 4 shows the wilted plants inoculated with *Rhizoctonia sp.* I and 22 (*a*) along with controls. Re-isolations from the infected plants yielded fungi with which they were inoculated.

In further experiments along these lines, the pots were replaced by tin cans with removable double bottoms, so that the roots for inoculation purposes could be exposed by washing from underneath without disturbing the plants. Results of two such experiments are summarised below :—

Fungus	Total plants inoculated	Total infected	Per cent infection
Rhizoctonia sp. I	23	4	17·39
Rhizoctonia sp. 22 (*a*)	27	16	59·26
Controls	30	0	0

Soil infection.—Sterilized soil was infected thoroughly with pure cultures of the fungi. Pots were filled with this soil and cotton sown in these. In the case of controls, no infection was added. Good germination and growth was obtained in control pots as well as those in which the soil had been infected with *Rhizoctonia sp.* 22 (*a*), while in the case of *Rhizoctonia sp.* I, the seed failed to germinate. Such seeds were found to be rotting and *Rhizoctonia sp.* I was readily isolated. Some germination, however, was obtained on third attempt at re-sowing of these pots. Plants in these pots were kept under observation for over three months and throughout this period, plants of different ages wilted from time to time and produced typical root-rot symptoms.

The fungus originally introduced was isolated.

To overcome the difficulty of poor germination due to rotting of the seed, the method of soil infection was modified as follows :—

The pots were filled to one-third of their depth with sterile soil, a layer about three inches in thickness of soil infected with pure cultures of the fungi was added and the rest of the pot filled with sterile soil. The object in view was that the seedlings should get a start before their roots passed into the infected zone.

Inoculations along these lines were carried out on a large scale and the disease was successfully reproduced in the pots which contained soil infected with the two *Rhizoctonia* species whereas the controls remained healthy.

Inoculation experiments in the field.—Trenches about three feet deep were dug along the rows of plants to be inoculated, taking care so as not to injure the roots. The roots of these plants were exposed by washing with a powerful spray hose. Pure cultures of the fungi grown on paddy were then introduced close to the roots and the trenches filled up with the soil that had been previously removed. The roots of plants in control rows were similarly exposed and covered up, but no fungal inocula were added.

To ensure that the wilting was due to the fungi with which the inoculations were carried out, re-isolations were made frequently and the fungi introduced were recovered.

Inoculation experiments in the field were carried out both with American (4-F) and Desi (*Mollisoni*) cottons using plants of varying ages. Results of five such experiments are given in Table V, which shows that the two species of *Rhizoctonia* are highly parasitic in nature.

TABLE V

Results of inoculation experiments carried out in the field with the two species of Rhizoctonia

Experiment	Inoculated with	No. inoculated	Variety	No. infected: Days after inoculation 2	3	4	5	6	7	8	9	10	11	12	13	14	Total infected	Per cent infection
I	*Rhizoctonia sp.* I .	21	*Mollisoni* .	1	..	4	1	2	..	..	..	..	..	..	1	..	9	42·85
	Rhizoctonia sp. 22 (*a*)	18	(7 weeks) .	2	3	2	..	..	..	..	..	..	..	..	..	..	7	38·88
	Controls . . .	19		ALL SOUND													0	0·00
II	*Rhizoctonia sp.* I .	44	*Mollisoni* .	9	16	2	..	..	1	..	..	..	1	..	..	..	29	65·90
	Rhizoctonia sp. 22 (*a*).	67	(7½ weeks).	..	7	2	..	..	2	..	..	..	..	..	..	..	11	16·41
	Controls . . .	44		ALL SOUND													0	0·00
III	*Rhizoctonia sp. I* . .	26	*Mollisoni* .	1	..	16	..	12 recovered						..	..	..	5	19·23
	Rhizoctonia sp. 22(*a*) .	43	(9 weeks) .	3	..	2	3	5 recovered						..	..	..	3	6·97
	Controls . . .	16		ALL SOUND													0	0·00
IV	*Rhizoctonia sp.* I .	10	4-F . .	3	1	..	..	..	..	..	..	..	..	..	..	..	4	40·00
	Rhizoctonia sp. 22(*a*) .	9	(12½ weeks)	..	2	..	..	..	..	..	..	..	..	..	..	..	2	22·22
	Controls . . .	10		ALL SOUND													0	0·00
V	*Phizoctonia sp.* I .	7	4-F . .	..	..	2	1	..	..	..	..	..	..	..	..	..	3	42·85
	Rhizoctonia sp. 22(*a*)	8	(15½ weeks)	..	..	1	..	..	..	..	..	..	..	..	..	..	1	12·50
	Controls . . .	7		ALL SOUND													0	0·00

Plate XXII, fig. 1 shows rows of plants which had wilted as result of inoculations with *Rhizoctonia solani* (I) and *Rhizoctonia bataticola* 22 (*a*). The rows of control plants are seen on the extreme right.

Plate XXII, fig. 2 shows an entire plant which had wilted due to inoculation with *Rhizoctonia solani* (I). Affected roots of the same plant are shown in Plate XXII, fig. 3.

Inoculations with *Rhizoctonia bataticola* 22 (*a*) produced similar symptoms.

The author wishes to thank Professor Rai Sahib Jai Chand Luthra for the keen interest and helpful suggestions throughout the course of this work. Thanks are also due to Mr. S. F. Ashby of the Imperial Mycological Institute, Kew, for help in the identification of the fungi.

X. Summary

1. Cotton root-rot is a very serious disease in the Punjab. The damage caused is estimated at Rs. 15,75,000 annually.

2. Symptoms of the disease are described.

3. The disease first appears in June and continues to be vigorous during July. In August the attack slows down and almost ceases by the end of September.

4. Several organisms were isolated from the diseased plants and inoculation experiments carried out in the pots as well as in the field by various methods. *Rhizoctonia solani* and another fungus, which is provisionally called *Rhizoctonia bataticola*, have been proved to be the causal organisms.

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FIG.1. Rows of cotton plants which had wilted as a result of inoculation with *Rhizoctonia* species I and 22 (*a*). Two rows of control plants are seen on the right.

FIG. 2. A cotton plant which had wilted on being inoculated with *Rhizoctonia* sp. I.

FIG. 3. Roots of 4 F cotton plant inoculated with *Rhizoctonia* sp. I.

STUDIES IN INDIAN CHILLIES

(4) INHERITANCE OF PUNGENCY IN *CAPSICUM ANNUUM* L.

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(Received for publication on 28th June 1934)

(With Plate XXIII)

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I. Introduction

The property for which the chilli crop is principally grown in India is the pungency of the pods. This pungent property is due to the presence in the fruit of a substance, called capsicin.

Pungency according to our investigations and those of Webber [1911] is definitely a heritable character. The degree of pungency, however, is found to vary considerably with soil and climatic factors. Erwin [1932] also maintains the view that the soil and climate considerably influence the degree of pungency of the chilli pod.

With regard to the mode of inheritance of this important character the results of the investigations of Webber [1911] show that the inheritance of this character is determined by a single factor. These results agree with our observations which also indicate that this character is monogenic.

II. Material and method

For the purpose of these investigations two Pusa types isolated by Shaw and Khan [1928], and since maintained pure, were selected as parents. Type 14 is

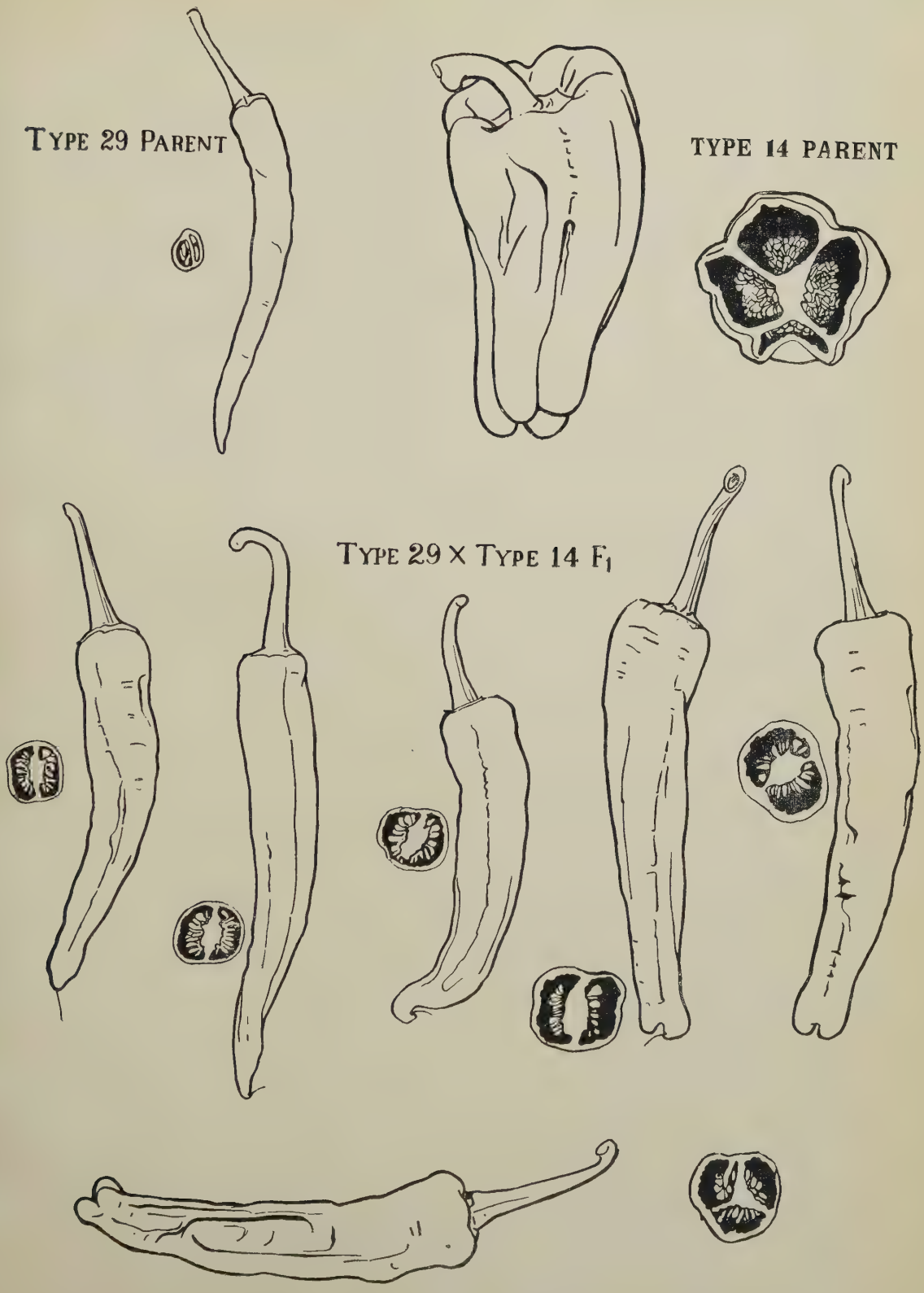
non-pungent while type 29 is pungent. The criterion of pungency was observation by taste by four independent workers. The crosses were attempted in the year 1928-29. A few fruits were obtained with type 29 as female parent but the reciprocal cross failed, and even in subsequent years this reciprocal cross was not successful.

The morphological characters in which the two parents differ are (1) the calyx enclosing or not enclosing the fruit-base, (2) the number of locules in the fruit, and (3) the fruit-apex, lobed or pointed (Plate XXIII). The inheritance of the first character had previously been studied in detail [Deshpande, 1933] in another cross and the character found to be monogenic, ' calyx not enclosing fruit-base ' being dominant to ' calyx enclosing fruit-base '. This character in the present cross was found to behave in the same way as in the previous cross and hence is not dealt with here at length but has been considered with pungency to see if any linkage exists between these two characters. The other two characters showed variation in the same plant in F_1 (Plate XXIII) and F_2 and their inheritance study had on this account to be dropped. Similar variation was observed in the previous cross [Deshpande, 1933] in the number of locules in fruits of the same plant in F_2 on which account this character was not further studied.

In F_2 and F_3 the plants were classified into two groups only, as ' pungent ' or ' non-pungent ' by actually tasting the fruits, though various degrees of pungency were suspected. This method was very trying and slow and it was impossible to examine more than about a dozen plants at a time. The free use of water and cane sugar helped to remove the pungent taste from the mouth but after tasting a very pungent fruit it was never possible immediately to diagnose correctly a less pungent fruit as ' pungent ' or a sweet fruit as ' non-pungent .' The doubtful cases had to be gone over again. In order to reduce the personal error the " tasting " was performed by four members of the staff and each diagnosis of pungency was made on these four independent observations. Due to these difficulties, it was not possible to examine the entire F_2 population, which numbered over 500 but we had to be limited to about 270 plants—which, for a monohybrid segregation, is a sufficiently big population. When this population was finally examined and classified the fruits of a few ' pungent ' and ' non-pungent ' plants were plucked at random and given for tasting to people who had not taken any part in the classification of the F_2 population. In every case the original diagnosis was confirmed. In F_3 all the plants of all the different cultures were examined.

III. F_1 GENERATION

The F_1 generation was grown in the year 1929-30 and consisted of 16 plants. The fruits of these plants were pungent but decidedly less so than the fruits of the pungent parent, type 29, thus proving that pungency is dominant (partially) to non-pungency. Out of these sixteen F_1 plants six were taken at random and selfed to grow the F_2 generation.



Variation in the fruit-apex and the number of locules in the same F_1 plant

IV. F_2 GENERATION

The F_2 generation was grown in the year 1930-31 and had a total population of over 500 of which the first 272 plants were examined and classified. The results are as follows :—

——	Pungent fruited plants	Non-pungent fruited plants	Total
Total observed.	202	70	272
Total expected on 3 : 1 basis . .	204	68	272
Ratio observed	2·97	: 1·03	
Ratio expected	3	: 1	

$$\frac{\text{Dev.}}{\text{P. E.}} = \frac{2}{4\cdot81} = 0\cdot416.$$

The fit, therefore, is very good.

V. THEORY

The above monohybrid segregation can be explained by assuming that **C** is the factor which is responsible for the production of pungency (Capsicin) in the chilli fruit and that when **C** is absent the fruit remains sweet or non-pungent.

The genetic constitution of the parents and the F_1 should, therefore, be as follows :—

Type 29 **CC**—Pungent

Type 14 **cc**—Non-pungent

Type 29 × Type 14 F_1 **Cc**—Pungent (partially)

The F_2 segregation should be on the mono-hybrid basis as follows :—

CC		**Cc**		**cc**
1	:	2	:	1
3 Pungent			:	1 Non-pungent

This result is realized in F_2

VI. F_3 GENERATION

The F_3 was grown in the year 1931-32. In all **twenty** F_2 plants were carried to the F_3 generation, and the behaviour of these in F_3 was as follows :—

No. of cultures in F_3 and nature of parent plant in F_2	Constitution of F_2 genotypes	Breeding behaviour in F_3	Frequencies	
			Observed	Expected
15 cultures from pungent fruited plants . .	**CC**	Pure	5	5
	Cc	Pungent Non-pungent 3 1	10	10
5 cultures from non-pungent fruited plants . .	**cc**	Pure	5	5

The F_3 observations, therefore, confirm the F_2 segregation on a mono-hybrid basis.

VII. Inheritance of pungency and the nature of calyx, enclosing or not enclosing fruit-base when both are considered together on di-hybrid basis.

It was ascertained in the previous investigations [Deshpande, 1933] that this calyx character behaves as a simple Mendelian character, 'calyx not enclosing fruit-base' being dominant to 'calyx enclosing fruit-base' and the F_2 observations in the present study confirmed the original results.

Considering the segregation of this character together with pungency, which also is a mono-genic character, we get the following di-hybrid segregation in F_2 :—

	Fruit pungent, calyx not enclosing fruit-base	Fruit pungent, calyx enclosing fruit-base	Fruit non-pungent, calyx not enclosing fruit-base	Fruit non-pungent, calyx enclosing fruit-base	Total
Total observed . . .	134	49	53	17	253
Total expected on 9 : 3 : 3 : 1 basis . . .	142·2	47·4	47·4	15·8	252·8
Ratio observed . . .	8·84	: 3·10	: 3·36	: 1·08	
Ratio expected . . .	9	: 3	: 3	: 1	

$X^2 = 1{\cdot}27$ and $P = 0{\cdot}739$

The fit, therefore, is good.

The above results clearly show that these two characters, pungency of fruits and calyx enclosing fruit-base, both of which have been contributed by one of the parents, type 29, are not linked but segregate independently.

VIII. Summary and conclusion

(1) Pungency is definitely a heritable character. The degree of pungency, however, is found to vary considerably with environment such as soil, climate, manurial treatment.

(2) The genetic results show that pungency is a simple monogenic character dominant to non-pungency and is determined by a single factor which we have termed **C**.

(3) 'Calyx not enclosing fruit-base' has been found to be dominant to 'calyx enclosing fruit-base' on a 3 : 1 basis.

(4) Both pungency and the nature of calyx, when studied together, have been found to segregate on di-hybrid basis each quite independently of the other.

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FHYLLODY : A POSSIBLE VIRUS DISEASE OF SESAMUM

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(Received for publication on the 18th May 1935)

(With Plates XXIV-XXVII)

The term phyllody is applied to a condition in plants wherein the floral organs are transformed into leaf-like structures. Phyllody is present every year to a greater or lesser extent in *til* (*Sesamum indicum* DC.) at Pusa and in some years as much as 15 per cent of the crop may be affected. As phylloid flowers do not set seed, phyllody, if present in appreciable proportions, may materially lower the yield.

Literature

That phyllody may be a disease of *til* seems to have been suspected as early as 1928 by Robertson [1928] in Burma where it is known as the " Phothe " or " green flowering " disease. He stated that in certain districts as much as 80 to 90 per cent of the plants may be affected, the symptoms manifesting themselves only in the flowering stage when the floral organs are changed into green leaf-like structures, accompanied by abnormally abundant branching.

Deighton [1931] observed a chlorosis attended by mottling and curling of leaves together with enations on the lower surface and inhibition of flowering in *Sesamum radiatum*, and Wallace [1933, 1934] reported a " leaf curl " which he considered to be a virus disease on *S. indicum*, but it is doubtful if the disease was phyllody. Storey [1933] whilst presenting no proof, considered the " green-flower " disease of *simsim* (*S. indicum*) to be also a virus disease but that he was referring to phyllody is apparent from the appropriate name by which the disease is designated. The " sepaloidy " in *til* reported by Kashi Ram [1930] and Roy [1931] is also, in all probability, phyllody. The former attributed the occurrence of the phenomenon to disturbed physiological conditions induced by early sowing and heavy rainfall. The second writer has not hazarded any opinion as to the cause of the abnormality.

Morphology

Affected *til* plants bear flowers wherein all the floral members except the stamens are either transformed into leaf-like organs or show a definite tendency to become leafy (Plate XXIV, figs. 2 and 3). The stamens retain their normal configuration but rarely contain functional pollen. In consequence of these changes the fertility of the plant is reduced and complete sterility may result.

The present observations are based on two *til* crops raised in the Botanical Section at Pusa in 1933 and 1934 respectively. Phyllloid plants cannot be distinguished from normal ones with certainty until flowering begins, *i.e.*, about 4-5 weeks after sowing in early varieties, and 5-7 weeks in late varieties. Phyllody may begin with the first flower in which case all subsequent flowers on the plant are phylloid or the change may occur later in the ontogeny in which event the first-formed flowers will be normal. In the latter type, phyllody manifests itself simultaneously at the tips of the main axis and the branches and in the new growth from the base so that it is not uncommon to see plants which bear capsules in the middle region but are phylloid at the extremities and at the base. No cases were encountered, however, in which some branches of the plant bore normal flowers exclusively and other branches, phylloid flowers; or in which the first formed flowers were phylloid and later ones normal.

Phyllody is always accompanied by a shortening of the upper internodes so that the abnormal flowers are crowded together and by a diminution in the size of the foliage leaves (in the floral region they are often lighter in colour); the zygomorphy which is well marked in the normal flower is lost and the phylloid flower, whether gamopetalous or polypetalous, is radially symmetrical. Glandular hairs are present on almost all parts of the sesamum plant but in the case of plants exhibiting phyllody these are found to occur as well in regions where they are normally absent, *i.e.*, the filaments and connectives of stamens, the style, stigma, inner surface of carpellary wall, and ovules. In the case of varieties which normally develop only one flower in the axil of each leaf (some varieties bear 2-3), the orange-yellow glands present on either side of the base of the flower stalk are replaced by small and phylloid, lateral, flowers, resulting in three flowers per axil.

The principal changes occasioned in the different parts of the flower, in phyllody, are briefly described below:—

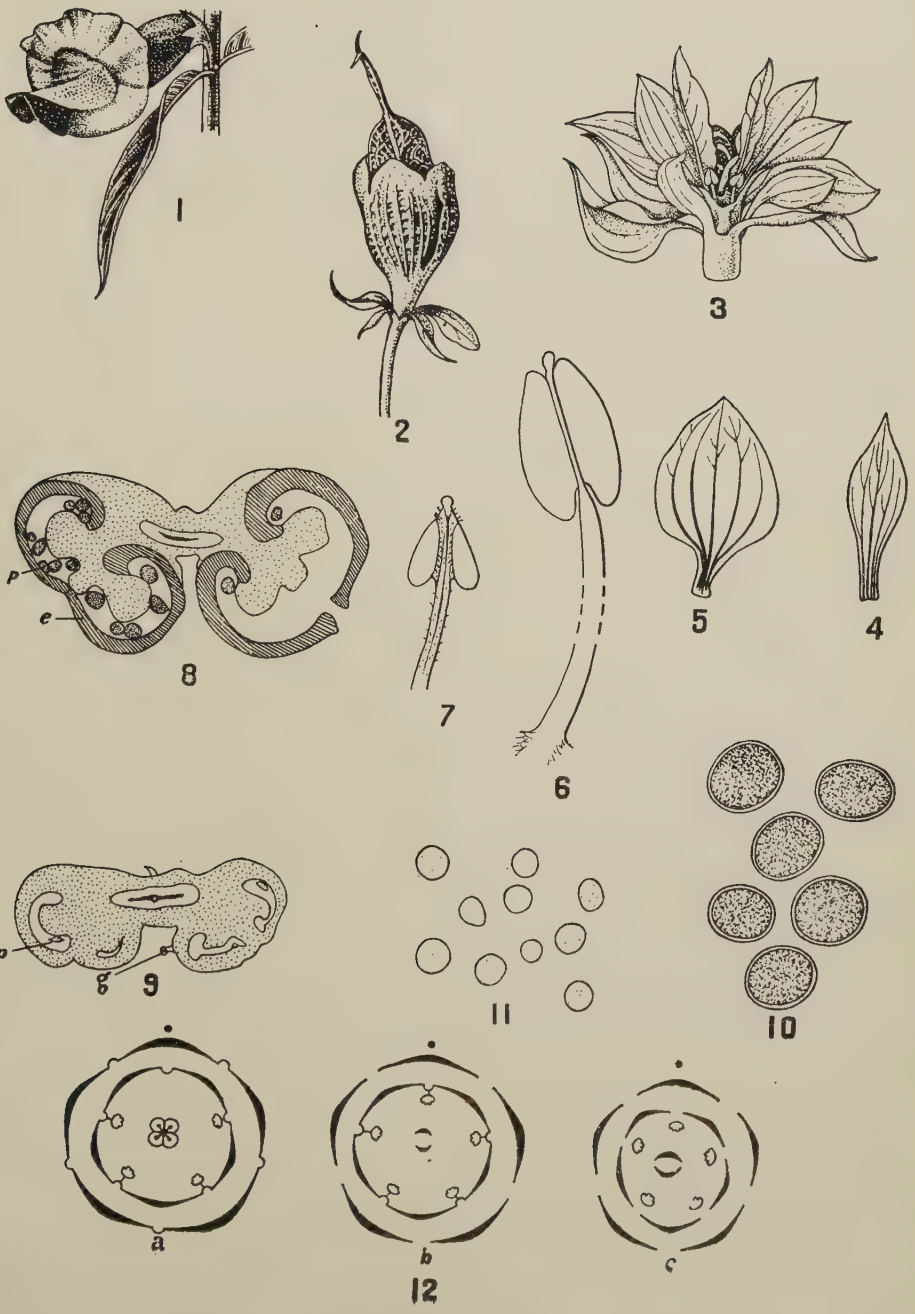
Calyx (*Plate XXIV, figs. 1-4*).—The normal calyx is green, gamosepalous, 5-partite, the segments being more or less linear, entire, with acute apices. In phylloid flowers, a complete division of the calyx tube occurs, resulting in a polysepalous calyx. The five sepals thus produced are sessile (occasionally sub-sessile), lanceolate or ovate-lanceolate, entire, with acute apices; they are rather leaf-like in appearance but are much smaller and possess a simpler configuration than foliage leaves. The venation is characteristic, the 3-7 primary veins being thickened and prominent. The internal structure of the phylloid sepal is similar to

Explanation of Plate XXIV.

1. Normal flower (⅔ natural size). 2. Partially phyllioid flower (⅔ natural size). 3. Totally phyllioid flower (×2). 4. Sepal of phyllioid flower (×2). 5. Petal of phyllioid flower (×2). 6. Normal stamen (×7). 7. Abnormal stamen; g=glandular hair (×7). 8. T. S. normal anther; e=endothecial layer; p=pollen grain (×28). 9. T. S. abnormal anther; g=glandular hair; p=pollen grain (×28). 10. Normal pollen grains (×108). 11. Pollen grains from abnormal anther (×108). 12. a, b, c. Floral diagrams of normal, partially phyllioid and totally phyllioid flowers.

Explanation of Plate XXIV.

1. Normal flower ($\frac{2}{3}$ natural size). 2. Partially phylloid flower ($\frac{2}{3}$ natural size). 3. Totally phylloid flower ($\times 2$). 4. Sepal of phylloid flower ($\times 2$). 5. Petal of phylloid flower ($\times 2$). 6. Normal stamen ($\times 7$). 7. Abnormal stamen; g=glandular hair ($\times 7$). 8. T. S. normal anther; e=endothecial layer; p=pollen grain ($\times 28$). 9. T. S. abnormal anther; g=glandular hair; p=pollen grain ($\times 28$). 10. Normal pollen grains ($\times 108$). 11. Pollen grains from abnormal anther ($\times 108$). 12. *a, b, c.* Floral diagrams of normal, partially phylloid and totally phylloid flowers.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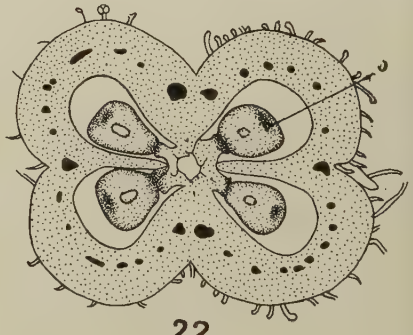

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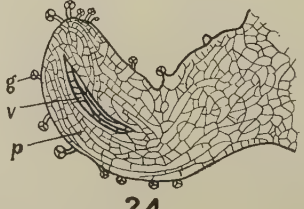

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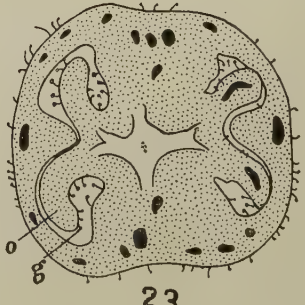

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Fig. 13. Normal ovary. (× 2)

,, 14-20. Modifications of ovary in phylloid flowers.
Figs. 14, 15, and 17, (×$\frac{4}{3}$); 18, 19, and 20 (×2); 16, ($\frac{2}{3}$ nat. size).

,, 21. Shoot arising from centre of phylloid flower and bearing "secondary" phylloid flowers. ($\frac{2}{3}$ nat. size).

,, 22. T. S. normal ovary. o=ovule. (×18)

,, 23. T. S. abnormal ovary. g=glandular hair, o=outgrowth replacing ovule. (×18)

,, 24. Outgrowth (o) in Fig. 23 enlarged to show lack of differentiation. g=glandular hair, p=parenchyma, v=vascular strand.

,, 25. Part of placenta from phylloid flower showing abnormal outgrowths produced in place of ovules. (×7)

Fig. 13. Normal ovary. (× 2)

" 14-20. Modifications of ovary in phyllioid flowers.
Figs. 14, 15, and 17, (×$\frac{2}{3}$); 18, 19, and 20 (×2); 16, ($\frac{2}{3}$ nat. size).

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" 24. Outgrowth (o) in Fig. 23 enlarged to show lack of differentiation. g=glandular hair, p=parenchyma, v=vascular strand.

" 25. Part of placenta from phyllioid flower showing abnormal outgrowths produced in place of ovules. (×7)

that of a foliage leaf but the venation is multicostate instead of being unicostate as in the latter.

Corolla (*Plate XXIV, figs. 1-3, 5*).—The margin of the corolla, or the whole of it, becomes green according to whether the phyllody is partial or complete; the surface is often rough, due to the veins being unusually thickened. Actinomorphic symmetry is acquired and the gamopetalous condition may be changed to a polypetalous one. Phylloid petals are similar in external appearance and internal structure to phylloid sepals except that the apices are rounded.

Andrœcium (*Plate XXIV, figs. 6-12*).—The stamens in the normal flower are four in number, epipetalous and didynamous. In the majority of abnormal flowers a fifth (anterior) stamen is developed and in completely phylloid flowers the stamens are no longer epipetalous (Plate XXIV, fig. 12). Though the stamens retain their normal configuration through all the stages of phyllody they are generally much smaller than normal ones and the anther lobes are usually reflexed. The whole stamen is green in colour.

In normal stamens there is a short connective and four pollen sacs, and the endothecial layer of fibrous banded cells which helps the sacs to dehisce is well developed (Plate XXIV, fig. 8); the pollen grains are dark-brown in colour, about 83—90 μ long and 77—83 μ broad. In phylloid flowers, the region of the connective is much broader, the epidermis shows a few glandular hairs and stomata, and the outer 2-3 cell-layers of both the connective and the pollen-sacs contain chloroplasts. The most distinctive feature, however, is the absence of the endothecial layer: this explains why the anthers do not dehisce. The cavity of the pollen-sac is greatly reduced by the excessive growth of parenchymatous tissue and is either empty or contains a few small (about 39—42 μ long and 33—39 μ broad), light-coloured, non-functional pollen grains in which the exine and intine are not differentiated.

Gynœcium (*Plate XXV, figs. 13-25*).—The chief modifications are (1) an enlargement of the ovary with the gradual loss of the style. The stigma may be transformed into two flattened, greenish structures. The ovary may become flattened and the veins of the carpellary wall are much thickened. (2) The gradual transformation of the carpellary wall to two foliaceous structures. In a few cases the latter are very similar in general configuration and venation to young foliage leaves but in the majority of cases a midrib is lacking and they are more like the phylloid sepals or petals.

The ovules are invariably non-functional in phylloid flowers. In the earliest manifestations of phyllody, a number of outwardly normal capsules may be produced but in these the ovules are replaced by parenchymatous outgrowths bearing glandular hairs. Often, however, small leaf-like structures are produced in place of ovules and in some cases the placenta forms a central axis on which such leaf like outgrowths are borne. In a few extreme cases, a central shoot grows out of

the middle of the flower, bearing phylloid flowers after the manner of the main inflorescence. These " secondary " phylloid flowers may in their turn give off shoots carrying " tertiary " phylloid flowers ; such cases, however, occur extremely rarely.

Flowers from 20 affected plants could be classified into the following nine classes :—

1. Sepals leaf-like ; corolla gamopetalous and green at margin ; ovary enlarged ; ovules non-functional.
2. Sepals leaf-like ; corolla gamopetalous, green ; ovary enlarged ; ovules non-functional.
3. Sepals leaf-like ; corolla gamopetalous, green ; ovary partially leaf-like ; ovules leaf-like.
4. Sepals leaf-like ; corolla gamopetalous, green ; ovary and ovules leaf-like.
5. Sepals leaf-like ; corolla polypetalous, green ; ovary partially leaf-like ; ovules leaf-like.
6. Sepals leaf-like ; corolla polypetalous, green ; ovary and ovules leaf-like.
7. Sepals leaf-like ; corolla polypetalous, green ; ovary leaf-like ; ovules absent.
8. Sepals leaf-like ; corolla gamopetalous, green ; ovary leaf-like ; ovules absent.
9. All parts except stamens leaf-like, crowded together in no apparent order.

The largest frequencies occurred in classes 6 and 9. None of the 30 Pusa sesamum types was found to be free from phyllody.

Cause

In an attempt to throw some light on the cause of phyllody the influence of the following factors on its incidence was investigated in 1934 : (1) time of sowing, (2) heavy manuring, (3) excessive humidity.

(*1*) *Time of sowing.*—To test the influence of this factor five sowings of two early *til* varieties (Pusa Types 3 and 7) were made at intervals of one week, starting from the 2nd August. The experiment was laid out in the form of randomised blocks.

The percentages of plants showing phyllody found in the five batches of sowings were 1·24, 1·58, 0·99, 0·25, 1·09 and 2·33, 1·73, 1·24, 0·34, 0·64, respectively for Type 3 and Type 7. The numbers in the earlier sowings are definitely, though not significantly, higher than those in the later sowings. As it is improbable that early sowing in itself can be responsible for the higher incidence of phyllody, it seems that the conditions obtaining at that time were more

FIG. 26. *a*. normal sesamum plant. *b*. phylloid sesamum plant.

FIG. 27. Sesamum plant which has become phylloid at a very late stage of growth. The tips of the main stem and branches (*a*) and the new shoots from near the base (*b*) are phylloid. The capsules (*c*) were produced by the first-formed normal flowers.

favourable for its occurrence. If a pathogene be concerned then the environmental conditions at the time of the earlier sowings may have been such as to promote its development and spread. It must also be remembered that the earlier the crop is sown, the longer it stands in the field, and, consequently, the longer it is exposed to possible attack.

(*2*) *Heavy manuring.*—Three treatments were tried, *viz.*, (*a*) Ammophos at 400 lb. per acre, (*b*) farmyard manure at 40 cart-loads per acre, and (*c*) control—no manure. The lay-out was again randomised blocks. The variety used was Type 7. No marked differences in number of phylloid plants per treatment resulted.

(*3*) *Excessive humidity.*—Sowings of Type 7 were made in three batches (at intervals of one week) in six lysimeters. Half the number of plants in each lysimeter was watered and covered with mats at night in order to promote highly humid conditions, this being done for a week, after the appearance of the first pair of foliage leaves ; the other half which served as a control was kept uncovered. In the day time, the mats were removed so as not to interfere with photosynthesis. There were 21 effective plants in each plot (*i.e.*, in each half-lysimeter). Out of the total of 252 plants in all the six lysimeters, however, only one exhibited phyllody.

Inoculation and grafting experiments

Inoculation and grafting were tried to see whether phyllody might be due to a virus. For the inoculation tests, twenty pairs of apparently normal plants were bagged and kept under observation for some time to make sure that they really were healthy. Pieces of phylloid shoots were then taken, washed thoroughly with sterilised water, crushed in a sterilised pestle and mortar, and the extracted juice injected by means of a hypodermic syringe into the stems of twenty plants. The remaining twenty plants which served as controls were injected with the same quantity of sterilised water. All the forty plants remained normal. As only one method of inoculation was attempted, however, it cannot be concluded that juice transmission is impossible.

At the same time as the above, six pairs of plants, each pair consisting of a normal and a phylloid plant, were taken and reciprocally grafted, *i.e.*, the top of the healthy plant (scion) was grafted on to the lower part of the phylloid plant (stock) and *vice versa*. In grafting, the region of the plant adjacent to the place of union was disinfected with absolute alcohol, and the scalpels, etc., employed were sterilised before and after use. Cleft grafting was performed and scion and stock were kept in position by a muslin bandage soaked in molten beeswax. Care was taken to seal the cut ends of the stalks of such leaves as were removed to facilitate grafting. After this the grafted plants were carefully bagged again. Unfortunately strong winds damaged most of the grafts and only four were successful—three grafts wherein the normal plant formed the stock and the phylloid plant the scion, and one of the opposite kind.

After about three weeks the originally normal scion of the last-mentioned grafts was found to be exhibiting symptoms of phyllody, and in the other three grafts, all new branches produced by the originally healthy stock bore phylloid flowers (Plate XXVII). Although the material is too scanty to enable definite conclusions to be drawn, these facts suggest that phyllody in sesamum is in the nature of a systemic disease and is possibly an addition to the growing list of plant viruses. Such an assumption is in consonance with the recorded observations on the incidence of phyllody and may explain, for example, why phyllody should be liable to reveal itself without apparent relation to the environmental conditions prevailing at the time.

Summary

Phylloid *til* plants are characterised by the transformation of all floral parts except the stamens into leaf-like structures. The different modifications encountered are briefly described.

An attempt was made to discover the cause of phyllody and it was found that neither heavy manuring nor the growing of plants under artificially contrived conditions of high humidity sensibly increased the percentage of phylloid plants. It was observed, however, that early sowings contained a larger proportion of them than late sowings.

Hypodermic injection of juice extracted from phylloid plants into the stems of normal plants gave negative results but it was found possible to transmit the phylloid condition by grafting normal scions on phylloid stocks and *vice versa*. This suggests—although the evidence is not conclusive—that phyllody may be caused by a virus.

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FIG. 28. Normal scion grafted on a phylloid stock. The former (*a*) is just showing symptoms of phyllody.

FIG. 29. Phylloid scion grafted on a normal stock. The new growth produced by the latter after grafting (*a*) shows obvious symptoms of phyllody.

STUDIES IN THE TECHNIQUE OF FIELD EXPERIMENTS

I. SIZE, SHAPE AND ARRANGEMENT OF PLOTS IN COTTON TRIALS

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(Received for publication on 5th June 1935)

(With one text-figure)

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Foreword

The development at the Institute of Plant Industry of an extensive experimental programme for fundamental research on cotton and for the amelioration of agricultural conditions in States in Central India and Rajputana has made necessary a number of investigations in statistical technique, and has provided opportunities for the study of a number of problems of design and analysis of experiments which are of general interest.

The work in progress falls naturally under two heads, first, the study of methods of increasing the accuracy of varietal trials for plant breeding purposes where rigorous scientific control is possible, and secondly, the development of a satisfactory simplified technique for trials carried out under non-technical control at out-stations or on cultivators' fields.

It is proposed to publish the results of these studies in a series of papers of which this is the first. This paper deals with investigations on the effect of size and shape of plots and blocks on standard error in replicated cotton trials. Further papers will deal with sampling for staple length measurement in cotton, the use of the method of covariance in breeding for disease resistance in cotton, and the effect of discarding margins on the accuracy of comparison in cotton and wheat trials.

J. B. H.

I. Introduction

Variety trials on cotton have been carried out by the Institute in Central India and Rajputana for the past three seasons using Latin Square and randomised block lay-outs. Plot sizes and shapes have been determined largely by convenience and such guidance as could be obtained from the literature on other crops. In 1933 it was decided to lay out a uniformity trial with Malvi mass-selected cotton at Indore in order to acquire more accurate information concerning the optimum conditions of plot size, shape and arrangement for cotton trials. The experiment was laid out at Indore chiefly for convenience of management, and conclusions concerning such matters as the magnitude of the standard error with plots of a certain size and shape are, of course, valid only for the conditions under which it was conducted. It is quite impossible to carry out such trials in all or even a number of the places where experimental work is to be done, but as experimental error has been of the same order in experiments carried out in various parts of Central India and Rajputana as at Indore, it is reasonable to suppose that conclusions reached at Indore concerning the optimum conditions of experimentation will be a fair guide for designing experiments in other areas.

II. Material

The experiment was carried out on a field of reasonably uniform fertility, so far as could be determined from inspection of crops of *jowar* (*Andropogon Sorghum*) and cotton grown in 1930 and 1931. The mean yield per acre of seed cotton from the experimental crop was 345 lbs. as against an average of 400 lbs. per acre given by the local control in ten varietal trials carried out on black soil in Malwa in 1932 and 1933. The field may, therefore, be classed as rather below average in fertility.

One hundred and thirty-eight rows each 206 ft. 8 in. long and 14 in. apart were sown north and south with a two-coultered seed dril (locally called *phadak*) at the end of June when rains began. Seed is dropped into the funnel of the *phadak* by hand, and a measured quantity of seed was sown in each row. The usual preliminary and post-sowing operations in practice at the Institute were carried out. At the time of harvest five rows on each margin of the field and ten feet at each end were discarded to avoid border effect. The central 128 rows were then divided into sections each four rows wide and 4 ft. 8 in. long. There were thus 1280 ultimate units, square in shape and 1/2000 acre in area. Four

pickings were made between the third week of November 1933 and the middle of January 1934. The data in this paper were calculated from the total yield (in grms.) of seed cotton per unit.

The units were combined to give plots 1, 2, 4 and 8 units broad and 1, 2, 4, 5, 8, 10, 20 and 40 units long. (Throughout the paper length will be used for length along the rows, and breadth, for breadth across the rows, irrespective of which dimension is the greater). These plots were grouped together (1) in blocks of four plots in a row (2) in blocks of eight plots in a row and (3) in blocks of eight plots in two rows of four.

By comparisons of different arrangements of units in plots, the effect of plot size and shape on plot error will be studied, and by comparisons of different numbers and arrangements of plots in blocks the relative efficiency of different block arrangements in removing variation due to major soil differences will be investigated.

III. Statistical analysis

In Table I are given percentage standard errors per plot for different plot sizes. The Table is subdivided into three sections to give standard errors per plot for the three types of block separately. In each case variation between blocks has been removed. The effect of this will be discussed below. It is sufficient to note here that the standard error is in general reduced by 50 to 70 per cent by the elimination of block differences (Table IV).

Table I

Standard errors per cent for plots of different sizes and shapes

Breadth in units	Length in units							
	1	2	4	5	8	10	20	40
				A. 4-plot blocks				
1	25·2	18·8	14·4	13·8	11·0	10·0	8·4	6·4
2	19·1	15·3	12·2	11·7	10·2	9·7	8·1	5·5
4	15·4	13·1	10·6	10·5	8·8	8·9	6·9	3·5
8	15·9	14·4	12·3	11·5	9·4	10·7	4·9	3·0
				B. 8-plot blocks in one row				
1	26·2	20·1	15·6	15·2	12·3	11·4	9·5	6·8
2	19·4	15·7	12·6	12·1	10·4	10·1	8·2	5·2
4	18·9	16·7	14·0	13·4	11·1	11·9	7·1	4·3
				C. 8-plot blocks in two rows				
1	25·9	20·1	17·2	14·3	..	17·4	11·9	..
2	20·1	16·3	15·0	11·8	..	16·8	10·8	..
4	16·6	14·0	13·5	10·5	..	16·1	9·5	..
8	16·4	13·9	13·2	10·8	..	15·0	8·3	..

It will be seen that there is a gradual decrease in percentage error per plot as the plot size increases by increase either in length or breadth; but with increase in length reduction in error is more rapid than with increase in breadth. The advantage of increased breadth rapidly diminishes until in the extreme case plots 8 units broad in Table I-A and 4 units broad in Table I-B are actually less advantageous than corresponding plots of half the breadth for all lengths except the two longest. Similarly plots 8 units broad in Table I-C show no advantage over corresponding plots of half the breadth. Table I thus brings out in general the advantage of bigger plots over smaller ones, and of long plots over broad ones. The standard errors per plot in 8-plot blocks are higher for all plot sizes than those in 4-plot blocks. When the 8 plots in a block were arranged in two rows instead of one, no advantage was secured over the single row arrangement and with plots 10 and 20 units long where the two-row arrangement was the less compact, it was actually less advantageous.

Tables II and III are arranged to demonstrate the effects of plot size and plot shape separately.

In Table II plots of different sizes but of the same shape are grouped together. For each shape, increase in plot size has consistently resulted in a decrease in standard error.

TABLE II

Standard errors per cent of plots of different sizes arranged according to shape

Plot shape (Breadth : Length)	Plot size (acre)	Per cent standard error per plot		
		4-plot blocks	8-plot blocks	
			1 row	2 rows
4 : 1	1/500	15·4	18·9	16·6
	1/125	14·4	..	13·9
2 : 1	1/1000	19·1	19·4	20·1
	1/250	13·1	16·7	14·0
	1/62·5	12·3	..	13·2

Plot shape (Breadth : Length)	Plot size (acre)	Per cent standard error per plot		
		4-plot blocks	8-plot blocks	
			1 row	2 rows
1 : 1 . . .	1/2000	25·2	26·2	25·9
	1/500	15·3	15·7	16·3
	1/125	10·6	14·0	13·5
	1/31·2	9·4	..	..
1 : 1·25 . . .	1/100	10·5	13·4	10·5
	1/25	10·7	..	15·0
1 : 2 . . .	1/1000	18·8	20·1	20·1
	1/250	12·2	12·6	15·0
	1/62·5	8·8	11·1	..
1 : 2·5 . . .	1/200	11·7	12·1	11·8
	1/50	8·9	11·9	16·1
	1/12·5	4·9	..	8·3
1 : 4 . . .	1/500	14·4	15·6	17·2
	1/125	10·2	10·4	..
1 : 5 . . .	1/400	13·8	15·2	14·3
	1/100	9·7	10·1	16·8
	1/25	6·9	7·1	9·5
	1/6·25	3·0	..	..
1 : 10 . . .	1/200	10·0	11·4	17·4
	1/50	8·1	8·2	10·8
	1/12·5	3·5	4·3	..
1 : 20 . . .	1/100	8·4	9·5	11·9
	1/25	5·5	5·2	..

In Table III plots of different shapes but of the same size are grouped together For each plot size, longer plots have in general given lower standard errors than broader plots.

TABLE III

Standard errors per cent of plots of different shapes arranged according to size

Plot size (acre)	Plot shape (Breadth : Length)	Per cent standard error per plot		
		4-plot blocks	8-plot blocks	
			1 row	2 rows
1/2000	1 : 1	25·2	26·2	25·9
1/1000	2 : 1	19·1	19·4	20·1
	1 : 2	18·8	20·1	20·1
1/500	4 : 1	15·4	18·9	16·6
	1 : 1	15·3	15·7	16·3
	1 : 4	14·4	15·6	17·2
1/400	1 : 5	13·8	15·2	14·3
1/250	8 : 1	15·9	..	16·4
	2 : 1	13·1	16·7	14·0
	1 : 2	12·2	12·6	15·0
	1 : 8	11·0	12·3	..
1/200	1 : 2·5	11·7	12·1	11·8
	1 : 10	10·0	11·4	17·4
1/125	4 : 1	14·4	..	13·9
	1 : 1	10·6	14·0	13·5
	1 : 4	10·2	10·4	..
1/100	1 : 1·25	10·5	13·4	10·5
	1 : 5	9·7	10·1	16·8
	1 : 20	8·4	9·5	11·9
1/62·5	2 : 1	12·3	..	13·2
	1 : 2	8·8	11·1	..
1/50	1·6 : 1	11·5	..	10·8
	1 : 2·5	8·9	11·9	16·1
	1 : 10	8·1	8·2	10·8
	1 : 40	6·4	6·8	..
1/31·2	1 : 1	9·4	..	..
1/25	1 : 1·25	10·7	..	15·0
	1 : 5	6·9	7·1	9·5
	1 : 20	5·5	5·2	..
1/12·5	1 : 2·5	4·9	..	8·3
	1 : 10	3·5	4·3	..
1/6·25	1 : 5	3·0	..	..

Plots of 20 units each were compiled in two ways, (1) with a breadth : length ratio of 1 : 20, and (2) with a breadth to length ratio of 20 : 1. Standard errors per cent per plot were calculated from the corrected plot yields and gave—

Along the rows (1 : 20) $\sigma = 10{\cdot}9$ per cent
Across the rows (20 : 1) $\sigma = 17{\cdot}6$ per cent
P (diff.) $= 0{\cdot}05$

Elimination of the fertility gradient, therefore, did not destroy the advantage of long plots over broad ones. Consideration of the method of sowing gives a reasonable explanation of the advantage, in that a measured amount of seed was sown in each row, and no doubt the individual dropping the seed into the *phadak* would tend to compensate any excesses in one section of the row by a deficiency in the next, and *vice versa*. This would result in greater variation between short lengths of the same row than between equal lengths of neighbouring rows. The longer the section of a row included in the plot, therefore, the less will be the variability in amount of seed sown per plot. In varietal trials an equal amount of seed is normally sown in each plot, and differences in seed rate will, therefore, not contribute to error. In agronomic trials, however, where seed is sown uniformly over a number of treatments, the arrangement of treatments to include long lengths of rows or even the whole rows may contribute materially to the reduction of error. A particular case is that in which each main plot in a confounded [Yates, 1933] experiment is divided in halves. Such divisions should wherever possible be along the rows and not across them.

In Table IV are given percentages of the total sums of squares removed by blocks of different sizes and shapes.

TABLE IV

Percentage variation removed by blocks of different sizes and shapes

4-plot blocks			8-plot blocks				
				One row		Two rows	
Block size (acre)	Block shape (Breadth : Length)	Percentage sum of squares	Block size (acre)	Block shape (Breadth : Length)	Percentage sum of squares	Block shape (Breadth : Length)	Percentage sum of squares
1/500	4 : 1	53·4	1/250	8 : 1	40·1	2 : 1	42·3
1/250	8 : 1	56·1	1/125	16 : 1	52·9	4 : 1	49·3
	2 : 1	61·9		4 : 1	49·1	1 : 1	49·3

4-plot blocks			8-plot blocks				
				One row		Two rows	
Block size (acre)	Block shape (Breadth : Length)	Percentage sum of squares	Block size (acre)	Block shape (Breadth : Length)	Percentage sum of squares	Block shape (Breadth : Length)	Percentage sum of squares
1/125	16 : 1	67·6	1/62·5	32 : 1	42·6	8 : 1	55·7
	4 : 1	66·2		8 : 1	58·6	2 : 1	55·4
	1 : 1	68·8		2 : 1	57·4	1 : 2	48·5
1/100	1 : 1·25	68·7	1/50	1·6 : 1	56·0	1 : 2·5	60·7
1/62·5	32 : 1	55·1	1/31·25	..	..	16 : 1	44·4
	8 : 1	70·3		16 : 1	43·4	4 : 1	60·1
	2 : 1	72·2		4 : 1	65·3	1 : 1	50·5
	1 : 2	72·7		1 : 1	60·2	..	..
1/50	1·6 : 1	71·3	1/25	3·2 : 1	64·3	1 : 1·25	66·3
	1 : 2·5	78·8		1 : 1·25	67·6	1 : 5	24·4
1/31·25	16 : 1	54·7	1/15·6	..	..	8 : 1	50·8
	4 : 1	75·4		8 : 1	50·6	2 : 1	53·7
	1 : 1	71·9		2 : 1	66·2	..	..
1/25	3·2 : 1	73·6	1/12·5	6·4 : 1	49·9	1·6 : 1	69·5
	1 : 1·25	77·3		1·6 : 1	70·7	1 : 2·5	19·4
	1 : 5	61·9		1 : 2·5	43·1	1 : 10	11·5
1/15·6	8 : 1	60·6	1/7·8	..	..	4 : 1	46·9
	2 : 1	76·2		4 : 1	55·5	..	..
1/12·5	6·4 : 1	61·2	1/6·25	..	..	3·2 : 1	59·7
	1·6 : 1	78·6		3·2 : 1	55·1	1 : 1·25	18·2
	1 : 2·5	55·1		1 : 1·25	46·4	1 : 5	6·2
	1 : 10	34·1		1 : 5	14·4	..	..
1/7·8	4 : 1	67·0	1/3·12	..	..	1·6 : 1	17·1
1/6·25	3·2 : 1	64·2		1·6 : 1	49·0	1 : 2·5	7·6
	1 : 1·25	58·6		1 : 2·5	21·9	..	..
	1 : 5	22·7					
1/13·12	2·6 : 1	70·4					

Table III also brings out the relative importance of size and shape of plots in reducing error. With the smaller plot sizes, the effect of size is predominant, so that larger plots are more efficient than smaller ones, irrespective of shape. With larger plot sizes, shape is considerably more important, so that broad plots are often less efficient than long plots of a smaller size. Plot shape is of most importance within the range of plot sizes usually employed in field experiments, and it is clear that the best use will be made of land if plots are made as long as is consistent with border row requirements and convenience of field arrangement.

It is a striking observation that in the present study, for any given plot shape, a considerably lower error was obtained when the longer dimension of the plot was along the rows than when it was across the rows. The most obvious explanation is that there may be a fertility gradient along the direction of sowing. A fertility contour map of the field was compiled from the yield data and is given in Fig. 1. Inspection shows that in general, and particularly on the western side of the field, a long plot running north and south (along the rows) will cross more of the fertility contour lines, and will therefore average out fertility differences better, than a plot of the same shape with its long dimension east and west. It was decided to take a square 20 units × 20 units from the northern half of the field (outlined in dotted lines in Fig. 1) and to correct the yields of the 400 units in it, for the regression of yield on position. The two regression coefficients were :—

N-S $b_1 = +2{\cdot}09$, $\sigma\ (b_1) = 0{\cdot}48$, $P = 0{\cdot}01$

E-W $b_2 = -0{\cdot}61$, $\sigma\ (b_2) = 0{\cdot}30$, $P = 0{\cdot}06$.

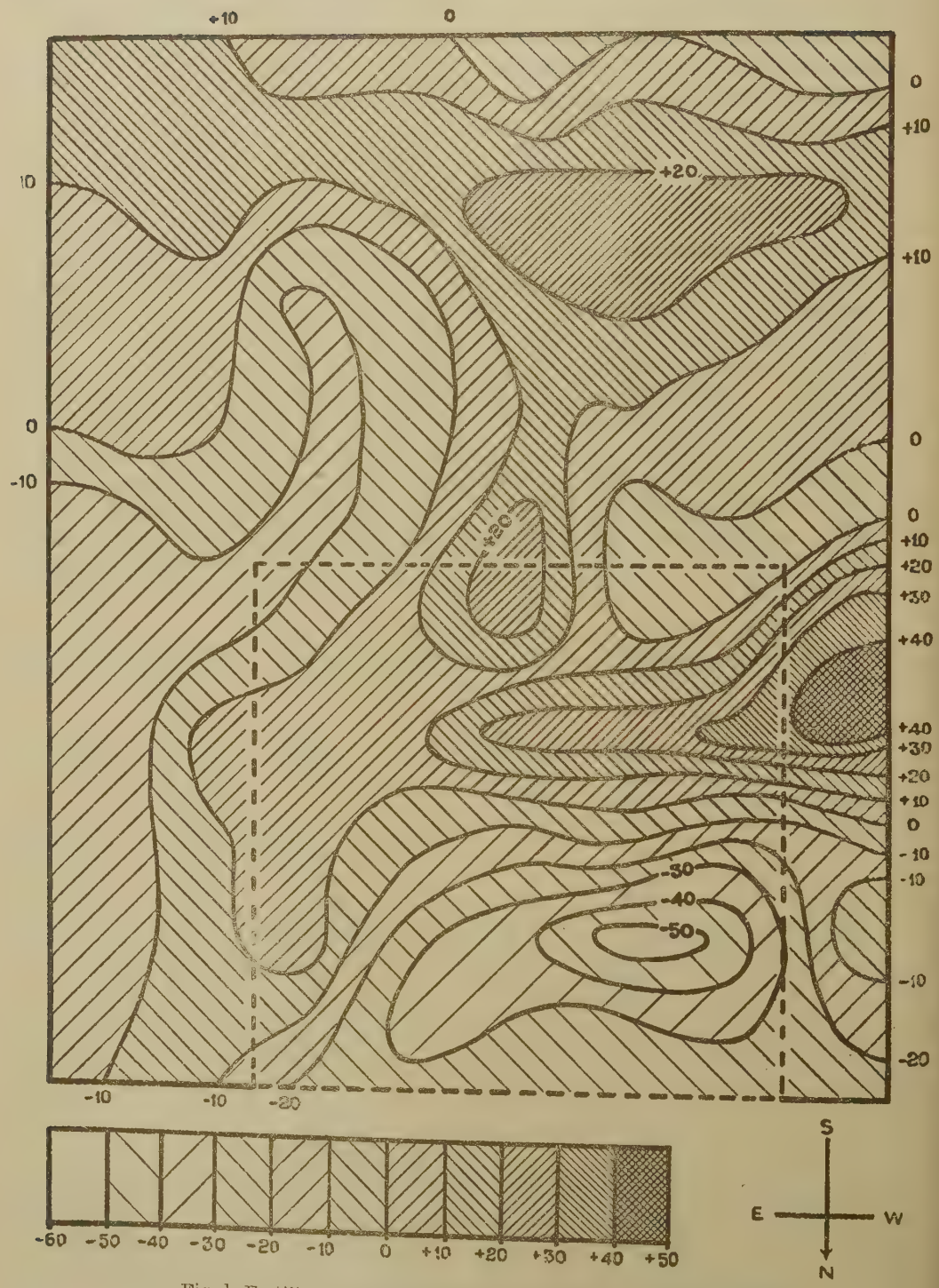

Fig. 1. Fertility contour map from data of cotton uniformity trial.

The failure of the Latin Square arrangement to give a lower error than an efficient randomised block arrangement may be ascribed to the fact that columns in the Latin Square represent blocks of a very inefficient shape. In the Latin Square arrangement, therefore, the number of degrees of freedom available for the estimation of error is reduced without any corresponding advantage. Eden and Fisher [1929], and Lord [1931] have previously discussed the failure of a Latin Square to give a lower error than randomised blocks.

IV. Discussion and conclusions

The drawing of conclusions concerning the conditions for efficiency in experimental lay-outs will be facilitated by a consideration of the nature of the statistical manipulations which have been carried out. A constant total variance—the variance between the 1/2000 acre units—has in every case been divided into three parts :—

(1) Variance included within plots.

(2) Variance between plots within blocks.

(3) Variance between blocks.

In experimental data item (1) does not appear and item (3) is eliminated in the statistical analysis. Item (2) remains as an estimate of plot error. The primary statistical consideration in experiment design is, therefore, to include as large a proportion as possible of the uncontrolled variability in items (1) and (3), and thereby to minimise item (2). Two important conclusions on this point have already been indicated, first, in order to maximise item (1), plots should be long and narrow, and secondly in order to maximise item (3), blocks should be approximately square.

Considerations of plot and block size are closely associated with matters of agricultural convenience, amount of land or seed available, and probable efficiency of supervision. It is therefore, desirable to present the conclusions in a manner which will enable the investigator to choose for himself the most efficient design consistent with the facilities available, rather than to attempt to define an optimum lay-out on statistical ground alone. With this aim in view the number of replications and the amount of land required to give a standard error of two per cent of the mean have been calculated for plots of different sizes and shapes, arranged in 4-plot and 8-plot blocks (Table VI). In the 8-plot blocks only the more efficient of the two arrangements of plots considered above has been included.

TABLE VI

Number of replications and area of land required to give a standard error of two per cent of the mean with different plot sizes and shapes

Plot size (acres)	Plot shape	4-plot blocks		8-plot blocks		
		No. of replications	Total area (acres)	Arrangement of plots	No. of replications	Total area (acres)
1/2000 . .	1 : 1	159	0·32	4 × 2	168	0·67
1/1000 . .	2 : 1	91	0·36	8 × 1	94	0·75
	1 : 2	88	0·35	8 × 1	101	0·81
1/500 . .	4 : 1	59	0·47	4 × 2	69	1·10
	1 : 1	58	0·46	8 × 1	62	0·99
	1 : 4	52	0·42	8 × 1	61	0·98
1/400 . .	1 : 5	48	0·48	4 × 2	51	1·02
1/250 . .	8 : 1	63	1·01	4 × 2	67	2·14
	2 : 1	43	0·69	4 × 2	49	1·57
	1 : 2	37	0·59	8 × 1	40	1·28
	1 : 8	30	0·48	8 × 1	38	1·21
1/200 . .	1 : 2.5	34	0·68	4 × 2	35	1·40
	1 : 10	25	0·50	8 × 1	32	1·28
1/125 . .	4 : 1	52	1·66	4 × 2	49	3·14
	1 : 1	28	0·89	4 × 2	45	2·88
	1 : 4	26	0·83	8 × 1	27	1·73
1/100 . .	1 : 1·25	27	1·04	4 × 2	27	2·16
	1 : 5	23	0·92	8 × 1	25	2·00
	1 : 20	18	0·72	8 × 1	22	1·76
1/62·5 . .	2 : 1	38	2·44	4 × 2	43	5·51
	1 : 2	19	1·22	8 × 1	31	3·97
1/50 . .	1.6 : 1	33	2·64	4 × 2	29	4·64
	1 : 2·5	20	1·60	8 × 1	35	5·60
	1 : 10	16	1·28	8 × 1	17	2·72
	1 : 40	10	0·82	8 × 1	11	1·76
1/31·2 . .	1 : 1	22	2·82	..	..	..
1/25 . .	1 : 1·25	29	4·64	4 × 2	56	7·95
	1 : 5	12	1·92	8 × 1	13	4·17
	1 : 20	7	1·12	8 × 1	7	2·24
1/12·5 . .	1 : 2·5	6	1·92	4 × 2	17	10·90
	1 : 10	3	0·96	8 × 1	5	3·21
1/6·25 . .	1 : 5	2	1·28	..	..	..

Four-plot blocks remove a larger proportion of the variation than eight-plot blocks of the same size and shape. In both four-plot and eight-plot blocks, blocks less than 1/100 acre remove a smaller proportion of the variation than blocks of 1/100 to 1/25 acre. Blocks of the latter range of sizes remove about 70 per cent of the variation in four-plot blocks and about 60 per cent in eight-plot blocks. With larger blocks a smaller proportion of the variation is removed and the proportion removed is largely affected by the shape of the block. With block sizes of 1/125 acre or less, block shape has little effect. As the block size increases above this point the efficiency of both long, narrow blocks and short, broad blocks rapidly decreases. With blocks of 1/25 acre or larger, the efficiency of all blocks with breadth greater than six times the length, or less than half the length, is very low.

Advantage was taken of the data collected to compare the efficiency of randomised block and Latin Square lay-outs. Two arrangements were taken, 64 plots arranged 8×8, each plot 1/100 acre in area, and $1 : 1 \cdot 25$ in shape, and 16 plots arranged 4×4, each plot 1/25 acre in area and $1 : 1 \cdot 25$ in shape. Analyses of variance are given below (Table V).

TABLE V

Analyses of variance of 8×8 and 4×4 Latin Squares

	Degrees of freedom	Sum of squares	Mean square
		8×8	
Rows	7	2,453,373	350,482
Columns	7	250,501	35,786
Residual	49	2,214,014	45,184
		4×4	
Rows	3	9,584,007	3,194,669
Columns	3	422,606	140,869
Residual	9	4,927,459	547,495

The mean square appropriate to error in the Latin Square arrangement is the residual mean square in each case. That appropriate to the randomised block arrangement can be obtained from Table V in two ways, by pooling with the residual sum of squares either the column or the row sum of squares and dividing by the appropriate number of degrees of freedom. It is immediately clear that the result obtained will be very different according to whether rows or columns are pooled with residuals. Below are given standard errors per cent per plot obtained as above for Latin Square and randomised block arrangements.

		8×8	*4×4*
Latin Square		13·6	11·8
Randomised blocks	Row-wise	13·4	10·7
	Column-wise	18·4	17·6

(Row-wise results from pooling columns with residual, and column-wise by pooling rows with residual.)

Clearly, if there is sufficient knowledge to design blocks in the most advantageous manner, randomised blocks can give as efficient a lay-out as a Latin Square. The required information is provided by the discussion of the influence of block shape on proportion of variation removed by blocks (*see* above). The percentages of the variation removed by blocks of the shapes constructed row-wise and column-wise are abstracted from Table IV and given below :—

Blocks	Block shape	Percentage variation removed
8-plot blocks, 1/12·5 acre		
Row-wise	6·4 : 1	49·9
Column-wise	1 : 10	11·5
4-plot blocks, 1/6·25 acre		
Row-wise	3·2 : 1	64·2
Column-wise	1 : 5	22·7

A standard error of two per cent of the mean was taken as a standard, a an investigator may reasonably demand experimental methods accurate enough to enable him to rely upon differences of the order of five or six per cent of the mean as significant. If differences of less than 11 per cent can safely be ignored, a standard error of 4 per cent will be sufficiently accurate, and the number of replications and the amount of land required will be one quarter of those given in Table VI.

The reduction in plot error with increasing plot size has already been pointed out. Table VI reflects this in the reduction in the number of replications required to give a 2 per cent error as plot size is increased. The reduction in error is not proportional to the increase in plot size, however, and consequently the amount of land required to give a two per cent error steadily increases as the plot size increases.

The effect of agricultural convenience, limitation of land or seed, and efficiency of supervision may now be considered.

Limited seed is available in the first stages of multiplication of selected strains in plant breeding work. Such limitation is usually confined to stages of work which are normally carried out on experiment stations where adequate supervision is available, and the expenditure of extra time and labour is of less importance than the acquisition, at an early stage, of reliable information concerning the potentialities of the strains under test. Experiments should, therefore, be laid out with small plots and many replications.

For the large majority of experiments carried out on experiment stations, plot size and number of replications can be varied within wide limits. In making a decision on design the following points of convenience should be borne in mind :

(1) Plots should be large enough to allow of the standard agricultural methods of the district being used, in order that the results obtained shall be strictly applicable to local conditions.

(2) Where land is limited, *e.g.*, where experiments are carried out on small areas irrigated from wells, smaller plots and more replications may be required.

(3) With very small plots the area occupied by marginal rows will be out of proportion to the whole. The full efficiency of small plots in reducing the area of land required cannot be obtained unless margins can safely be dispensed with.

The design of experiments for out-stations requires special study. It may be stated here, however, that where skilled supervision is lacking or intermittent, simplicity in design is essential. For this, plots should be large and replications comparatively few. Thus the number of separate plot products to be harvested and weighed is minimised, and the risk of having to weigh small lots on crude scales is avoided. Margins and guard rows should form as small a proportion of the whole as possible.

In Institute experiments it has been usual to employ plots of 1/100 acre with a breadth/length ratio of 1 : 2. With six replications a standard error of 4 per cent of the mean would be expected. In actual fact standard errors have ranged from 5 to 10 per cent of the mean, depending on efficiency of supervision and facilities for accurate execution. In practice, therefore, it may be expected that rather more replications, and rather larger areas will be required for a given standard error than are indicated in Table VI, since there are likely to be sources of error in a multiple plot experiment which do not exist in a uniform crop not subdivided until harvest time. Considerable improvement in accuracy may confidently be expected if plot size is increased from 1/100 acre to 1/50 acre and the plots are made longer in proportion to their breadth.

V. Summary

1. The results of a uniformity trial with Malvi cotton at Indore are reported.

2. Standard error per cent per plot decreased steadily with increasing plot size.

3. For any given plot size standard error per cent per plot decreased steadily as the length of the plot (along the rows) was increased.

4. The advantage of long, narrow plots laid out along the rows over plots of the same size and shape laid out across the rows is shown to be independent of fertility gradient, and it is suggested that it is associated with the method of sowing.

5. It is shown that approximately square blocks eliminate more of the soil heterogeneity than rectangular ones. With blocks of 1/25 acre or larger, shape is the most important factor in determining efficiency.

6. A Latin Square lay-out has no advantage over an efficiently designed randomised block lay-out with the same number of plots.

7. Optimum lay-outs for different types of experimentations are discussed and a table is given to facilitate determination of the amount of land and number of replications required for a given accuracy with different plot sizes.

VI. Acknowledgments

Our thanks are due to Mr. Y. D. Wad, under whose charge the experiment was laid down, before the senior author's arrival, and to whose interest and criticism we have been indebted throughout.

A large part of the laborious computation involved was undertaken by Mr. S. A. Khargonekar, whose assistance we take this opportunity of acknowledging.

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INHERITANCE OF CHARACTERS IN SORGHUM—THE GREAT MILLET

VII. LIGULE AND AURICLE

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(Received for publication on 3rd April 1935)

(With Plate XXVIII)

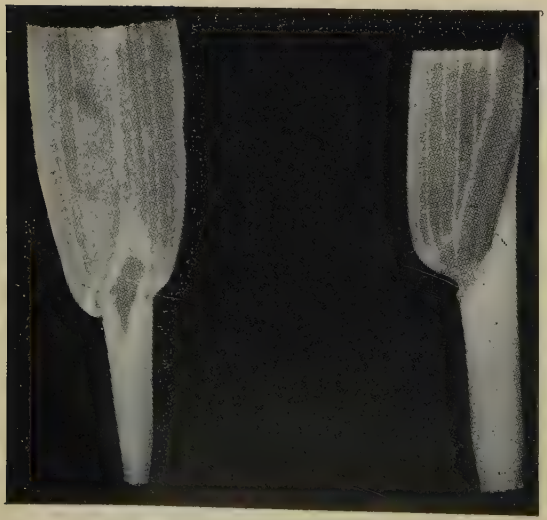
In sorghum, as in all grasses, there is a well-marked zone at the junction of the leaf sheath and the leaf blade. This zone has two parts, (1) a narrow ligule which is a prolongation of the distal inner margin of the leaf sheath, and (2) a triangular, light coloured membranous tissue, which is usually flat, in sorghum, and may vary in the width of its base. In extreme cases as in *Sorghum caudatum* Stapf, the length of the base may be so great as to throw the outline into a curve of a broad sweep giving it an auricular disposition in which the lobes meet and encircle the stem. These structures and their variations are related both to leaf size and leaf disposition and have varietal peculiarities. It will be obvious that the specialised zone at the junction of the leaf blade and leaf sheath plays an important role in the development of the leaf blade by making it possible for the latter to widen out and link up with the clasping leaf sheaths.

A condition in which both the ligule and the junction are absent, though uncommon, has been recorded in the wild grasses as well as in the cultivated cereals. Emerson [1912] first noted this condition in maize and recorded that it behaved as a simple recessive to the presence of ligule and auricle. Vavilov and Bukinich [1929] have noted it as a simple recessive condition in wheat. Brink [1933] finds that two factors are responsible for the ligulate and auriculate condition the absence of either or both of which would give an e-ligulate and non-auriculate plant.

At the Millets Breeding Station, two instances have been met with of this e-ligulate and non-auriculate condition, one in Sudan grass and the other in Broom corn, both being relatively primitive types of sorghum. These forms have probably arisen as mutants from normal ligulate populations and being peculiar and recessive were easily picked out and perpetuated. Pure lines of these e-ligulate and non-auriculate plants were grown at Coimbatore and in both of them some natural crosses occurred possessing both ligule and auricle. Six of these were taken for study and the seedlings raised from them showed clear segregations for the ligulate and auriculate condition and its absence.

NX No.	F_2 seedlings	
	Ligulate and auriculate	E-ligulate and non-auriculate
Sudan grass		
A. S. 3695	320	102
Broom corn—		
A. S. 3696	545	174
„ 3697	322	108
„ 3698	266	79
„ 3699	243	78
„ 3700	236	71
Total (observed)	1,932	612
Total (calculated)	1,908	636

$\chi^2 = 1 \cdot 21$, $P > \cdot 1$.



Ligulate and auriculate

E ligulate and non-auriculate

It will be seen that this e-ligulate and non-auriculate condition is due to the absence of a single factor that behaves as a simple recessive to its presence. Whether more than one factor is involved in this phenomenon only wider experiences can show. It will be seen from the illustration (Plate XXVIII) that in the absence of the auricular adaptation, the seedlings have their leaves erect and close in marked contrast to the free and wide disposition of the normal seedlings. The adult plants also showed this difference. This appearance of a primitive character, probably as a result of a new environment, is interesting as throwing light on the evolution of the ligule and auricle. Moreover in maize a linkage has been established between the factor for ligule and the **Rg** factor for raggedness in the blade [Brink and Senn, 1931]. The normal ligulate and auriculate character has been designated **Lg** to bring it into line with the nomenclature used for maize.

Summary

An e-ligulate and non-auriculate condition has been met with in the leaves of sorghum. This character has been designated **lg** , the normal ligulate and auriculate condition being **Lg**. **Lg** is a simple dominant to **lg**.

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NOTES.

ROUND-TABLE DISCUSSION ON NUTRITION AT THE NEXT CONGRESS OF THE FAR EASTERN ASSOCIATION OF TROPICAL MEDICINE

We have received the following circular letter from the local Secretary of the Far Eastern Association of Tropical Medicine :—

At the next Congress of the Far Eastern Association of Tropical Medicine, it is proposed to hold a round-table discussion on nutrition, and we have been asked by the Council to make preparation for it.

Papers are invited upon Nutrition from the widest point of view and we should be glad if you will be so good as to ask trained observers who are working on any aspect of the subject in your country, whether they will kindly contribute to the discussion by reading a paper on their work under any of the sub-headings below.

If suitable support is forthcoming, it may be possible to combine the papers received and the discussions in a volume, which would constitute an up-to-date account of Nutrition as concerns the East.

It is hoped that some indication of the support which may be expected from your country may be received during 1935 though it will not be necessary for titles of papers to be sent in until a later date which will be notified in due course. Such co-operation will enable the Council to know how much time should be allotted for the discussion.

It has been proposed to divide papers under three headings as follows :—

I. Economics.—To include such aspects as agriculture in relation to human nutrition, *e.g.*, improvement of yield and quality of food crops ; horticulture ; fruit-growing ; stock-raising ; dairy problems ; institutional feeding ; food surveys ; storage ; cooking, etc.

II. Chemical and physiological.—To include food analyses in the widest sense ; vitamin, mineral, fat, protein studies, etc.; metabolism, basal metabolism, energy requirements, specific dynamic action.

III. Clinical.—Studies of disease in relation to food and diet, the feeding of infants during the first year with special reference to development (height and weight) ; children's diseases in relation to food ; nutritional œdema, atypical beriberi ; the course of infectious diseases

under the influence of food ; liver cirrhosis ; anæmias ; skin diseases in relation to food and vitamins ; ulcers of the leg ; leprosy in relation to food ; constitutional diseases ; diabetes ; obesity ; gallstones ; gastric ulcer, etc.; clinical value of certain foods, etc.

It should be understood that the above provisional programme is intended to be as wide as possible, and that additional suggestions from those able to make them will be welcomed. It is hoped that the subject of nutrition will receive emphasis from the general and normal point of view as well as from the point of view of disease.

IMPORTATION OF 'MEXICAN JUMPING BEANS' INTO BURMA

In exercise of the powers conferred by sub-section (i) of section 3 of the Destructive Insects and Pests Act, 1914 (II of 1914), the Governor General in Council has been pleased to direct that the following further amendment shall be made in the Order published with the Notification of the Government of India in the late Department of Revenue and Agriculture, No. 580-240, dated the 22nd June 1922, namely :—

Paragraph 8 of the said Order shall be re-numbered " 8(a) " and after the paragraph as so re-numbered the following sub-paragraph shall be inserted namely :—

" (b) The importation of ' Mexican Jumping Beans ', (*Sebastiania palmeri* of the family Euphorbiaceae is prohibited absolutely."

GIPD—61 IC of AR—23-8-35—800.